EBV CONFERENCE 2008

2008
EBV CONFERENCE

The 13th Biennial Conference of The International Association for Research on Epstein-Barr Virus and Associated Diseases

Final Program and Abstracts

中山大學肿瘤防治中心
SUN YAT-SEN UNIVERSITY CANCER CENTER

7th-10th November
Guangzhou, China

Welcome

Welcome to Guangzhou and the 13th Biennial Conference of the International Association for Research on Epstein-Barr Virus and Associated Diseases! It is a great honor for us to hold this EBV conference just after 2008 Beijing Olympic Game.

As one of the biggest city in China, Guangzhou is enriched in history, education and culture. Being in history for more than 2,800 years, Guangzhou has a great deal of legends related to the important events in Chinese history, such as “Campaign to suppress the opium in 1838 led by Zexu Lin”, “the Revolution of Sun Yat-sen”, and “the Openning up of Xiaoping Deng”. In addition, Guangzhou is a flourishing and rich city, as its GDP is ranked at the third among all cities in China. Notably, Medical Science in Guangzhou is also on the top 5 list in China mainly due to the Sun Yat-sen University School of Medicine. For example, Sun Yat-sen University Cancer Center, the host for this conference, has over 10,000 patients per year.

Flowers are blooming everywhere and all the year round in Guangzhou, and there are a lot of tourist attractions, such as White Cloud Hill Scenic Spot, Yuexiu Park, Dr. Sun Yat-sen Memorial Hall, Conghua Hot Springs, and Xiqiao Hill Scenic spot. Cantonese cuisine is one of 8 famous foods in China. Specially, the delicate Cantonese pastry is well-known for its wide range of varieties, plus delicious flavor and beautiful color. That’s why people say “Go Eating in Guangzhou”.

The organization of this Conference has been supported by a very helpful local committee and a wonderful international board of advisers. Thanks to all of you, we have a bunch of science to be presented in the meetings. This Symposium shall continue to advance our knowledge in EBV field. I hope that it will be a scientific Olympic Game.

On behalf of the Organizing Committee, I welcome you to Guangzhou once again, and will ensure that all of you enjoy the meeting and our city.

With best Regards
Yin-xin Zeng, M.D., Ph.D.
Chair, Organizing Committee
Director, Sun Yat-sen University Cancer Center
Guangzhou, China

Chairman

Professor Yi-Xin Zeng
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Friday, November 7

09:00-19:00 **Registration**
19:00-21:00 **Reception and Opening Performance**

Saturday, November 8

Invited Presentation 1 **Saturday, November 8**
Chairperson: Ingemar Ernberg **08:15-08:55**

08:15-08:55 1 EARLY EVENTS IN PRIMARY B CELLS INFECTED WITH EPSTEIN-BARR VIRUS
Wolfgang Hammerschmidt

Session 1: LATENT INFECTION (Ⅰ) 7~10 **Saturday, November 8**
Chairpersons: Alan Rickinson, George Sai-Wah Tsao **08:55-10:15**

08:55-09:15 7 EPISOME STABILITY OF EBV IS REGULATED BY AN S-PHASE CHECKPOINT THAT DELAYS REPLICATION TIMING
Jing Zhou, Andy Snyder, and **Paul M. Lieberman**

09:15-09:35 8 CELL GENE REGULATION ASSOCIATED WITH EBER EXPRESSION
Goran Gregorovic, Daniel Charnock, Oliver Dittrich-Breiholz, Michael Kracht, Rainer Russ and Paul J. Farrell

09:35-09:55 9 THE REGULATION OF EPSTEIN-BARR VIRUS LATENT GENE EXPRESSION BY CYTOKINES
Loránd L. Kis, Daniel Salamon, Emma Persson, Noémi Nagy, George Klein, and Eva Klein

09:55-10:15 10 EXPERIMENTAL RHESUS MACAQUE INFECTION WITH A RHESUS LYMPHOCRYPTOVIRUS MOLECULAR CLONE.
Makoto Ohashi, Angela Carville, Carol Quink and Fred Wang

10:15-10:30 **Morning Coffee**

Invited presentation 2 **Saturday, November 8**
Chairperson: Fred Wang **10:30-11:10**

10:30-11:10 2 EBNA1 INHIBITS DNA REPAIR AND APOPTOSIS IN NASOPHARYNGEAL CARCINOMA THROUGH THE DISRUPTION OF PML NUCLEAR BODIES
Lori Frappier

Session 2: LATENT INFECTION (Ⅱ) 11~14 **Saturday, November 8**
Chairpersons: Friedrich Grasser, Kenzo Takada **11:10-12:30**

11:10-11:30 11 ESTABLISHMENT OF STABLE INFECTION OF EPSTEIN-BARR VIRUS IN PREMALIGNANT NASOPHARYNGEAL EPITHELIAL CELLS
GSW Tsao, CM Tsang, G Zhang, PM Hau, C Man, YL Yip,LL Sheung，E. Seto，K Takada，Y Cao，Chen HL, KW Lo, DY Jin, DLW Kwong

11:30-11:50 12 BZLF1 PROMOTER SILENCING ELEMENTS ZV/ZV' AND ZIIR FUNCTION SYNERGISTICALLY IN ESTABLISHING AND MAINTAINING EBV LATENCY
Xianming Yu, Patrick J. McCarthy, Richard J. Kraus, Amy Ellis, Zhenxun Wang, Hui Jun Lim, and Janet E. Mertz

11:50-12:10 13 INTERACTION OF EXOGENOUS AND ENDOGENOUS VIRUSES IN HEAD AND NECK CARCINOMA
Semyon Rubinchik, Jacob Smith, Brian Hoel, Lucinda Halstead, David White, Marion B. Gillespie, and **Natalie Sutkowski**

12:10-12:30 14 METHYLATION GENERATES FUNCTIONALLY DIFFERENT SUBSPECIES OF THE EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN2
Henrik Gross, Alfredo Mamiani, Stephanie Barth, Christine Hennard, Ursula-Zimber-Strobl, Elisabeth Kremmer, and **Friedrich A. Grasser**

12:30-13:40 **Box Lunch and Poster Viewing**

Session 3: VIRAL REPLICATION 15~19 **Saturday, November 8**
Chairpersons: Alison Sinclair, Paul Farrell **13:40-15:20**

13:40-14:00 15 THE EPSTEIN-BARR VIRAL PROTEIN EB2 STRONGLY STIMULATES TRANSLATION OF INTRONLESS RNAS INDEPENDENTLY OF PKR
Emiliano Ricci, Fabrice Mure, Henri Gruffat, Didier Décimo, Cahora Medina-Palazon, Théophile Ohlmann and **Evelyne Manet**

14:00-14:20 16 MURINE GAMMAHERPESVIRUS-68 ORF33 ENCODES A TEGUMENT PROTEIN ESSENTIAL FOR VIRION EGRESS
Haitao Guo and **Hongyu Deng**

14:20-14:40 17 HIGH EXPRESSION LEVELS OF STAT-3 ARE CHARACTERISTIC OF CELLS REFRACTORY TO EPSTEIN-BARR VIRUS LYTIC CYCLE INDUCTION
Derek Daigle, Cynthia Megyola , Ayman El-Guindy, Lyn Gradoville, George Miller, Sumita Bhaduri-McIntosh

14:40-15:00 18 GENE SPECIFIC ACTIVATION AND RNA BINDING BY EBV SM PROTEIN
Zhao Han, Dirk Dittmer and **Sankar Swaminathan**

15:00-15:20 19 EPSTEIN-BARR VIRUS COLONISATION OF B CELL SUBSETS IN PRIMARY INFECTION AND PERSISTENCE
S. Chaganti, A. Bell, C. Ma, D. Croom-Carter, E. Heath, S. Tangye, A. Hislop, W. Bergler, M. Kuo, M. Buettner, G. Niedobitek and A.B. Rickinson

15:20-15:40 **Afternoon Coffee**

Session 4: GENETICS, NON-CODING RNAS AND EPIGENETIC **Saturday, November 8**
Chairpersons: Paul Ling, Ingemar Ernberg 20~24 **15:40-17:20**

15:40-16:00 20 EPIGENETIC ETIOLOGY OF NASOPHARYNGEAL CARCINOMA
Qian Tao

16:00-16:20 21 MIR-BART5 IS AN ANTI-APOPTOTIC MIRNA ENCODED BY EBV AND INHIBITS PUMA TO PROMOTE HOST CELL SURVIVAL
Elizabeth Yee-Wai Choy, Ka-Fai To, Dora Lai-Wan Kwong, Sai Wah Tsao and **Dong-Yan Jin**

16:20-16:40 22 EBV LATENCY IN B CELLS LEADS TO EPIGENETIC REPRESSION AND CPG METHYLATION OF THE TUMOUR-SUPPRESSOR GENE BIM
Kostas Paschos, Paul Smith, Emma Anderton, Jaap M Middeldorp, Robert E White and Martin J Allday

16:40-17:00 23 NOVEL TUMOR SUPPRESSOR GENES ASSOCIATED WITH INITIATION AND PROGRESSION OF NASOPAHRYNGEAL CARCINOMA
Ning Wang, Tanya Pavlova, Lin Mo, Veronika Zabarovska, Zhiming Du, Vladimir Kashuba, Guangwu Huang, Maria Lung, Ingemar Ernberg, Eugene Zabarovsky and **Lifu Hu**

17:00-17:20 24 ABILITY OF ZTA TO INTERACT WITH METHYLATED ZRES IN HUMAN AND VIRAL PROMOTERS: ROLE IN OVERTURNING EPIGENETIC SILENCING?
Questa H. Karlsson, Celine Schelcher, Elizabeth Verrall, James Heather, Kirsty Flower, Carlo Petosa and **Alison J. Sinclair**

17:20-19:00 **Dinner**

19:00-22:00 **Poster Viewing**

19:00-21:00 **Round Table Meeting for EBV Vaccine**

Sunday, November 9

Session 5: SIGNAL TRANSDUCTION (Ⅰ) 25~30 **Sunday, November 9**
Chairpersons: Lawrence Young, Martin Allday **08:25-10:25**

08:25-08:45 25 MECHANISTIC STUDY OF THE LMP1-MEDIATED NON-CANONICAL NF-KAPPAB PATHWAY
Po-yan Cheung and **Zhenguo Wu**

08:45-09:05 26 MOLECULAR BASIS OF CYTOTOXICITY OF EPSTEIN-BARR VIRUS (EBV) LATENT MEMBRANE PROTEIN 1 (LMP1) IN EBV LATENCY III B CELLS: LMP1 INDUCES TYPE II LIGAND-INDEPENDENT AUTOACTIVATION OF CD95/FAS WITH CASPASE 8-MEDIATED APOPTOSIS.
Christophe LE CLORENNEC,: Le Clorennec C, Ouk TS, Youlyouz-Marfak I, Panteix S, Martin CC, Rastelli J, Adriaenssens E, Zimber-Strobl U, Coll J, Feuillard J, Jayat-Vignoles C

09:05-09:25 27 PROTEOMIC IDENTIFICATION OF THE TYROSINE PHOSPHATASE SHP1 AS A NOVEL LMP1 INTERACTION PARTNER WHICH MEDIATES AUTOREGULATION OF LMP1 SIGNALING
Janine Griese , Thomas Knfel, Helmut Kutz, Stephan M. Feller, **Arnd Kieser**

09:25-09:45 28 REGULATION OF MAPK MEDIATED OP18/STATHMIN SIGNALING PATHWAY TRIGGERED BY EBV ENCODED LMP1 IN NASOPHARYNGEAL CARCINOMA CELLS
Xuechi Lin, Sufang Liu, Lili Guo, Zijian Li, Xiaoqian Ma, Lili Li, Xiyun Deng, **Ya Cao**

09:45-10:05 29 INDUCTION OF EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 BY ENDOPLASMIC RETICULUM STRESS IN NASOPHARYNGEAL CARCINOMA CELLS
Yao Chang, Jenn-Ren Hsiao, Chaio-Wei Chen, Shih-Yi Wu, Mei-Chi Hsu, and Ih-Jen Su

10:05-10:25 30 THE SRC FAMILY KINASE, LYN, NEGATIVELY REGULATES AKT ACTIVATION IN LMP2A-EXPRESSING CELLS
Jillian M. Brandon, Stephanie E. Whittaker, Diana J. Windsor, Sorcha A.M. Collins, and **Robert J. Ingham.**

10:25-10:40 **Morning Coffee**

Session 6: SIGNAL TRANSDUCTION (Ⅱ) 31~34 Sunday, November 9
Chairpersons: Yao Chang, Takeshi Sairenji 10:40-12:00

10:40-11:00 31 EBV-ENCODED EBNA-5 BINDS TO MDM2 AND MDMX AND PREVENTS P53-INDUCED GROWTH ARREST AND APOPTOSIS
E. Kashuba, M. Yurchenko, SP. Yenamandra, B. Snopok, B. Bercovich, A. Ciechanover, and G. Klein

11:00-11:20 32 EPSTEIN-BARR VIRUS-ENCODED EBNA1 MODULATES THE NF-KB PATHWAY IN CARCINOMA CELLS IN VITRO
R. Valentine, K. M. Shah, J.R. Arrand, C.W. Dawson, L.S. Young and **J.D O'Neil**

11:20-11:40 33 NF-KB SUPPORTS GLYCOLYSIS IN EBV TRANSFORMED LCLS
Thomas Sommermann and **Ellen Cahir-McFarland**

11:40-12:00 34 THE EBV DEUBIQUITINATING ENZYME, BPLF1, INTERACTS WITH AND REDUCES THE ACTIVITY OF EBV RIBONUCLEOTIDE REDUCTASE.
Christopher B. Whitehurst, Julia Shackelford, Edward Gershburg, Gretchen L. Bentz, and Joseph S. Pagano

12:00-13:40 **Box Lunch and Poster Viewing**

12:30-13:40 **EBV Association Board Meeting**

Invited Presentation 3 **Sunday, November 9**
Chairperson: Ya Cao **13:40-14:20**

13:40-14:20 3 EBV SEQUENCE VARIATION AND NPC
Yi-xin Zeng, Tiebang Kang

Session 7: NASOPHARYNGEAL CARCINOMA 35~39 **Sunday, November 9**
Chairpersons: Kai-tai Yao, Qian Tao **14:20-16:00**

14:20-14:40 35 MASSIVE OVER-EXPRESSION OF CHITINASE3-LIKE4 (YM2), CD30 AND IMMUNOGLOBULIN IN LMP1 INDUCED INFLAMMATION-ASSOCIATED CARCINOGENESIS
Adele Hannigan, P. Monica Tsimbouri and **Joanna B. Wilson**

14:40-15:00 36 NASOPHARYNGEAL CARCINOMA (NPC): MOLECULAR PATHOGENESIS
Chin-Tarng Lin

15:00-15:20 37 FUNCTIONAL CHARACTERIZATION OF A CHROMOSOME 11 CANDIDATE TUMOR SUPPRESSOR GENE, THY1, IN NASOPHARYNGEAL CARCINOMA
HL Lung, AKL Cheung, Y Cheng, FM Kwong, PHY Lo, EWL Law, D Chua, ER Zabarovsky, N Wang, SW Tsao, EJ Stanbridge, **ML Lung**

15:20-15:40 38 MOLECULAR GENETIC ANALYSIS OF NASOPHARYNGEAL CARCINOMA
Chunfang Hu, Xiaoyi Chen, Wenbin Wei, Andrew I Bell, John Nicholls, Yunhong Yao, Irène Joab, Paul G Murray, Lawrence S Young, Chris W Dawson and **John R Arrand**

15:40-16:00 39 BMI-1 INDUCES EMT AND METASTASIS IN PNC
Li-bing Song, Jun Li, Wen-ting Liao, Yan Hong, Yi-xin Zeng and **Mu-sheng Zeng**

16:00-16:15 **Afternoon Coffee**

Invited Presentation 4 **Sunday, November 9**
Chairperson: Nancy Raab-Traub **16:15-16:55**

16:15-16:55 4 EBV AND HODGKIN'S LYMPHOMA; NEW INSIGHTS INTO AN OLD ASSOCIATION
Paul Murray

Session 8: BURKITT'S LYMPHOMA 40~43 **Sunday, November 9**
Chairpersons: Evelyne Manet, Denis Moss **16:55-18:15**

16:55-17:15 40 AN EPSTEIN-BARR VIRUS ANTI-APOPTOTIC PROTEIN CONSTITUTIVELY EXPRESSED IN TRANSFORMED CELLS AND INVOLVED IN BURKITT LYMPHOMAGENESIS: THE BAMHI W PROMOTER/BHRF1 CONNECTION
Gemma L. Kelly, Heather M. Long, Julianna Stylianou, Wendy A. Thomas, Andrew I. Bell, Georg Bornkamm, Josef Mautner, Alan B. Rickinson and Martin Rowe

17:15-17:35 41 EFFECTS OF HOLOENDEMIC MALARIA ON PRIMARY EBV INFECTION IN CHILDREN FROM WESTERN KENYA
Erwan Piriou, Amolo Asito, Nancy Fiore, Peter S. Odada, Jaap M. Middeldorp, Ann M. Moormann, Robert Ploutz-Snyder, **Rosemary Rochford**

17:35-17:55 42 THE EPSTEIN-BARR VIRUS BART MIRNAS PROVIDE A SURVIVAL ADVANTAGE TO BURKITT'S LYMPHOMAS
David Vereide and Bill Sugden

17:55-18:15 43 CO-TRANSPLANTED HUMAN THYMUS IMPROVES CONTROL OF EPSTEIN-BARR VIRUS INFECTION IN A HUMANIZED-SCID-GAMMA C-/- MOUSE MODEL, AND LYTIC VIRAL INFECTION INCREASES LYMPHOMA NUMBER.
Shidong Ma, Deepika Rajesh, Ruth Sullivan, William J. Burlingham, Xiaoping Sun, Margaret L. Gulley, and Shannon C. Kenney

18:30-21:00 **Pearl River Evening Cruise (Dinner on boat)**

Monday, November 10

Invited Presentation 5 **Monday, November 10**
Chairperson: Alison Sinclair **08:15-08:55**

08:15-08:55 5 EPTEIN-BARR VIRUS-INDUCED ONCOGENESIS THROUGH UTILIZING THE INNATE IMMUNITY SYSTEM
Kenzo Takada

Session 9: OTHER MALIGNANCIES AND PTLD 44~46 **Monday, November 10**
Chairpersons: Shannon Kenney, Lori D Frappier **08:55-09:55**

08:55-09:15 44 THE INVESTIGATION OF INTERACTION BETWEEN NASAL NK/T-CELL LYMPHOMA AND THE INFLAMMATION CELL.
Hideyuki Ishii, Miki Takahara, Yasuaki Harabuchi, Eva Klein

09:15-09:35 45 MECHANISMS UNDERLYING THE SELECTIVE IMPAIRMENT OF EBNA1-SPECIFIC EFFECTOR T-CELLS OBSERVED IN PTLD.
K. Jones, J. Nourse,S. Yekollu, L. Morrison, D. Moss, MK Gandhi

09:35-09:55 46 CLINICOPATHOLOGIC FEATURES AND EBV GENOME POLYMORPHISM OF EPSTEIN-BARR VIRUS-ASSOCIATED GASTRIC CARCINOMA IN GUANGZHOU
Chun-Kui Shao, Jian-Ning Chen, Yun-Gang Ding

09:55-10:10 **Morning Coffee**

Session 10:IMMUNE MECHANISMS, VACCINE AND IMMUNOTHERAPY **Monday, November 10**
Chairpersons: Maria Masucci, Jaap Middeldorp 47~53 **10:10-12:30**

10:10-10:30 47 ACTIVATION AND EXPANSION OF PROTEIN-SPECIFIC T-CELLS BY UREA FORMULATED PROTEINS OR PEPTIDES: IMPLICATION FOR DIAGNOSTIC, PREVENTIVE AND THERAPEUTIC APPLICATIONS
Deml, L.; Barabas, S.; Edmaier, K.; **Wolf, H.**

10:30-10:50 48 EPSTEIN-BARR VIRUS-ENCODED BILF1 CONTRIBUTES TO IMMUNE EVASION BY TARGETING MHC CLASS I FOR DEGRADATION
Jianmin ZUO, Andrew CURRIN, Wendy A THOMAS and Martin ROWE

10:50-11:10	49	BLOOD DIFFUSION AND TH1-SUPPRESSIVE EFFECTS OF GALECTIN-9 CONTAINING EXOSOMES RELEASED BY EPSTEIN-BARR VIRUS-INFECTED NASOPHARYNGEAL CARCINOMA CELLS Jihène Klibi, Toshiro Niki, Alexander Riedel, Catherine Pioche-Durieu, Mitsuomi Hirashima, Fethi Guemira , Sylvestre Le Moulec , Joël Guigay , Dinesh Adhikary, Josef Mautner, **Pierre Busson**
11:10-11:30	50	THE EFFECTS OF ACUTE MALARIA ON EBV LOAD AND EBV-SPECIFIC T CELL IMMUNITY IN GAMBIAN CHILDREN **R. Njie,** A. Bell, H. Jia, D. Croom-Carter, S. Chaganti, A. Hislop, H. Whittle and A. Rickinson
11:30-11:50	51	BARF1 AS NEW IMMUNOTHERAPEUTIC TARGET FOR NASOPHARYNGEAL CARCINOMA D. Martorelli, K. Houali, L. Caggiari, E. Vaccher, L. Barzan, G. Franchin, A. Gloghini, A. Pavan, A. Da Ponte, R.M. Tedeschi, V. De Re, A. Carbone, T. Ooka, P. De Paoli, **R. Dolcetti.**
11:50-12:10	52	STAGE-SPECIFIC INHIBITION OF MHC CLASS I PRESENTATION BY THE EPSTEIN-BARR VIRUS BNLF2A PROTEIN DURING VIRUS LYTIC CYCLE **Nathan CROFT,** Claire SHANNON-LOWE, Andrew BELL, Danielle HORST, Maaike RESSING, Emmanuel WIERTZ, Martin ROWE, Alan RICKINSON, Andrew HISLOP
12:10-12:30	53	NEWLY SYNTHESISED POLYPEPTIDES VERSUS MATURE PROTEIN AS ENDOGENOUS ANTIGEN SOURCES FOR CD4+ AND CD8+ T CELL RECOGNITION : STUDIES WITH EBNA1 AND EBNA3B **Mackay L.K**., Long H.M., Brooks J.M., Mautner J. & Rickinson A.B
12:30-14:00		**Box Lunch and Poster Viewing**
13:30-14:00		**EBV Association General Meeting**

Henle Lecture **Monday, November 10**
Chairperson: Sankar Swaminathan **14:00-14:40**

14:00-14:40	6	THE MOLECULAR BIOLOGY OF EBV: FROM B95 TO NPC **Nancy Raab-Traub**

Session 11: DIGNOSTICS, DRUG DEVELOPMENT AND THERAPEUTICS **Monday, November 10**
Chairpersons: Stephen Gottschalk, Kwok-Wai Lo **54~56** **14:40-15:40**

14:40-15:00 54 IMAGING EBV-ASSOCIATED NASOPHARYNGEAL CARCINOMA IN VIVO
De-Xue Fu, Yvette Tanhehco, Jianmeng Chen, James Fox, Catherine Foss, Sridhar Nimmadda, George Sgouros, Martin Pomper and Richard Ambinder

15:00-15:20 55 HUMORAL IMMUNE RESPONSES TO EPSTEIN-BARR VIRUS ENCODED TUMOR ASSOCIATED PROTEINS AND THEIR PUTATIVE EXTRACELLULAR DOMAINS IN SERA FROM NASOPHARYNGEAL CARCINOMA PATIENTS AND REGIONAL CONTROLS.
Dewi K. Paramita, Christien Fatmawati, Hedy Juwana, Frank G. van Schaijk, Jajah Fachiroh, Sofia M. Haryana, and **Jaap M. Middeldorp**

15:20-15:40 56 HSP90 INHIBITORS INDUCE KILLING OF EPSTEIN-BARR VIRUS-POSITIVE TUMOR CELLS AND REDUCE EXPRESSION OF EBNA1
Xiaoping Sun, Shidong Ma, Elizabeth Barlow, Shannon Kenney

15:40-16:00 **Afternoon Coffee**

16:00-17:00 **Poster Talks (Selected 4-6 posters)**

17:00-17:30 **Closing Remarks**

17:30-21:00 **Banquet**

21:00 **Bus to Ocean Hotel (located in the central city) for people who stay after the meeting**

After Meeting Activity (optional):

Guangzhou City one day tour (optional, fees included in registration for international participator)
Tuesday (Nov. 11): 8:30 to 17:00

Poster sessions

Poster session 1: Latent Infection 57~72

57 EPSTEIN-BARR VIRUS BART GENE EXPRESSION
Maha Al-Mozaini, Gustavo Bodelon, Claudio Elgueta Karstegl, Boquan Jin, Mohammed Al-Ahdal and Paul J. Farrell.

58 INVOLVEMENT OF HSC70 AND EBV LATENT MEMBRANE PROTEIN-1 IN THE REGULATION OF CELL MITOSIS
Robert Qi

59 A SYSTEM BIOLOGY APPROACH FOR THE ROLE OF EBNA3C IN MODULATING THE S-G2 JUNCTION OF CELL CYCLE.
Tathagata Choudhuri

60 PRIMA-1MET INDUCES NUCLEOLAR TRANSLOCATION OF EPSTEIN BARR VIRUS-ENCODED EBNA-5 PROTEIN
György Stuber1, Emilie Flaberg1, 2, 3, Gabor Petranyi1, 2. 5., Rita 謙 v 鰛 1, Nina R 鰊 aeus4, Elena Kashuba1, 3, Klas G. Wiman4, George Klein1 and Laszlo Szekely1, 2, 3

61 CHANGES IN THE CHEMOKINE AND CHEMOKINE RECEPTOR EXPRESSION AFTER EPSTEIN-BARR VIRUS INFECTION OF TONSILLAR B CELLS
Barbro Ehlin-Henriksson1,,Liang Wu1, Alberto Cagigi1, Frida Mowafi1, George Klein1 and Anna Nilsson1,2

62 INTERFERON REGULATORY FACTORS 4 IS INVOLVED IN EPSTEIN-BARR VIRUS-MEDIATED TRANSFORMATION OF HUMAN B LYMPHOCYTES
Dongsheng Xu 1, Lingjun Zhao 2, Luis Del Valle3, Judith Miklossy3, and Luwen Zhang 1

63 IDENTIFICATION OF DELTANP63 AS A MODULATOR OF LMP1 EXPRESSION IN EBV-INFECTED EPITHELIAL CELLS
J.Jia, C.W. Dawson and L.S.Young

64 EBNA-LP RELOCALIZES SMRT/NCOR AND HDAC5 FROM MAD BODIES TO THE NUCLEOPLASM
Daniel Portal And Elliott Kieff

65 AN IN VITRO MODEL FOR LATENCY IN THE GAMMAHERPESVIRUSES
K. Mutyambizi (1, 2), H. Coleman (1), V. Connor (1), S. Efstathiou (1)

66 **EBV INDUCES AN ATM AND CHK2-DEPENDENT DNA DAMAGE RESPONSIVE PATHWAY EARLY AFTER INFECTION THAT LIMITS B CELL PROLIFERATION AND LONG-TERM LCL OUTGROWTH**

Micah Luftig, Eleonora Forte, Olena Rusyn, Pavel Nikitin, Chris Yan, David Tainter, Jing Guo

67 **BZLF1-KNOCKOUT EBV TRANSFORMS PRIMARY B-LYMPHOCYTES AS EFFICIENTLY AS WILD-TYPE EBV**

K.R. Katsumura, S. Maruo, K. Takada. Institute for Genetic Medicine, Sapporo, Japan

68 **EBNA-1 PROMOTES GENOMIC INSTABILITY BY INDUCING THE PRODUCTION OF ROS**

Bettina Gruhne, Ramakrishna Sompallae, Diego Marescotti, Siamak Akbari Kamranvar, Stefano Gastaldello and Maria G. Masucci

69 **THE EPSTEIN-BARR VIRUS ONCOPROTEIN, LATENT MEMBRANE PROTEIN-1 REPROGRAMS TONSILLAR B CELLS TOWARDS A HODGKIN'S REED STERNBERG LIKE PHENOTYPE**

Martina Vockerodt,1 Katerina Vrzalikova1,2, Susan L Morgan,1 Wenbin Wei,1 Dieter Kube,3 Lawrence S Young, 1 Ciaran B Woodman,1 and Paul G Murray1

70 **EBV LMP2A MODULATES THE TYROSINE KINASE SYK TO INDUCE INVASION IN EPITHELIAL CELLS**

Chen F, Gish G1, Ingham RJ1,2, Werner M, Pawson T1,3, Ernberg I*

71 **EBNA 2 STIMULATES TRANSCRIPTIONAL ELONGATION THROUGH PTEFB AND SPT5**

Richard Palermo, Helen Webb and Michelle J. West

72 **FINDING THE REGULATORS OF EPSTEIN-BARR VIRUS LATENCY**

Omid R. Faridani, Annette Dirac, Christian Gentili, Rene Bernards, Maria G. Masucci

Poster Session 2：Viral Replication **73~98**

73 **CRYSTAL STRUCTURE OF EBV DNASE**

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74 **REGULATION OF EPSTEIN-BARR VIRUS BZLF1**
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Beverly E. Griffin1, 4, Susan van Noorden2, I.A. Lampert2, S-A Xue3 and J.A. Phillips4. Imperial College School of Medicine at St. Mary's1 and at Hammersmith Hospitals2, University College Medical School3, London U.K., and the BL unit, Kamuzu Central Hospital, Malawi4

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Jiang Li1,2,3, Hao-yuan Mo4, Fang Qiu4, Yi-Nan Zhang1,2,3, Qiu-Yan Chen4, Yong-qiang Li1,2,3, Li-Xi Huang1,2,3, Maria G Masucci5# and Yi-Xin Zeng1,2,3#

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Frank G. van Schaijk, Iedan Verly & Jaap M. Middeldorp, Dept. Pathology, VU University Medical Center, Amsterdam, The Netherlands

214 **DYNAMIC ANALYSIS OF EPSTEIN-BARR VIRUS IN THE PROCESS OF NASOPHARYNGEAL CARCINOMA THERAPY**

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Jade Yee, Goran Gregorovic, Paul J Farrell, Robert E White and Martin J Allday, Imperial College London

217 **SUBEROYLANILIDE HYDROXAMIC ACID (SAHA) INDUCES VIRAL LYTIC CYCLE IN EPSTEIN-BARR VIRUS (EBV) - INFECTED GASTRIC CARCINOMA CELLS AND MEDIATES ENHANCED CELL DEATH**

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220 **TWO STEP EPSTEIN - BARR VIRUS IGA-ELISA SYSTEM FOR SEROLOGICAL SCREENING AND CONFIRMATION OF NASOPHARYNGEAL CARCINOMA.**

Dewi K. Paramita1, Jajah Fachiroh1,Sofia M. Haryana1 and Jaap M. Middeldorp2.

221 **HETEROGENEOUS RIBONUCLEOPROTEIN K AND THYMIDINE PHOSPHORYLASE ARE INDEPENDENT PROGNOSTIC AND THERAPEUTIC MARKERS FOR NASOPHARYNGEAL CARCINOMA**

Lih-Chyang Chen, Chuen Hsueh, Ngan-Ming Tsang, Ying Liang, Kai-Ping Chang, Sheng-Po Hao, Jau-Song Yu, Yu-Sun Chang

222 **STUDY ON EPSTEIN-BARR VIRUS AND HUMAN HERPES VIRUS 6 INFECTION IN DRUP ERUPTION PATIENTS**

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223 **GANCICLOVIR MEDIATED INHIBITION OF EBV REPLICATION REQUIRES THE VIRALLY ENCODED KINASE (EBV-PK)**

Qiao Meng, Stacy Hagemeier, Shannon C. Kenney

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224 **THE SRC FAMILY KINASE, LYN, NEGATIVELY REGULATES AKT ACTIVATION IN LMP2A-EXPRESSING CELLS**

Jillian M. Brandon, Stephanie E. Whittaker, Diana J. Windsor, Sorcha A.M. Collins, and Robert J. Ingham

Invited speaker
Abstract

1 (RegID: 1068)
Wolfgang Hammerschmidt
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EARLY EVENTS IN PRIMARY B CELLS INFECTED WITH EPSTEIN-BARR VIRUS

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Abstract:
In *ex vivo* isolated human B cells EBV establishes an exclusively latent infection. Very much in contrast to most other herpesviruses (with the exception of KSHV) synthesis and release of progeny virus does not take place initially. This block of de novo virus synthesis seems to be tight, but infectious virus becomes readily detectable several weeks post infection. The molecular basis of this phenomenon is unclear. In latently infected primary B cells, the two viral lytic switch genes, BZLF1 and BRLF1, are expressed early after infection similar to the viral Bcl-2 homologues (vBcl-2) BHRF1 and BALF1 (Altmann and Hammerschmidt, PLoS Biol 2005, 3(12):e404). The early expression of vBcl-2 genes is essential for the transformation of infected human B cells but the role of BZLF1 and BRLF1 early after infection remains unclear. Here I concentrate on the analysis of the early phase of infection, which we termed EBV's abortive lytic phase, and its role in the process of B cell transformation and establishment of latency.

2 (RegID: 1838)
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EBNA1 INHIBITS DNA REPAIR AND APOPTOSIS IN NASOPHARYNGEAL CARCINOMA THROUGH THE DISRUPTION OF PML NUCLEAR BODIES

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Abstract:
The EBNA1 protein of EBV plays several important roles in EBV latent infection including activating the replication and mitotic segregation of EBV episomes and transactivating the expression of other EBV latency genes. In addition, increasing evidence indicates that EBNA1 can affect the cellular environment in a way that might contribute to cell immortalization and/or malignant transformation. Using proteomics methods, we have identified interactions between EBNA1 and several host proteins including the key cell cycle regulators, ubiquitin specific protease 7 (USP7) and casein kinase 2 (ck2), suggesting that EBNA1 modulates cellular processes through sequestration of host proteins. Recently we have examined the cellular effects of EBNA1 in nasopharyngeal carcinoma (NPC) cells to assess possible contributions of EBNA1 to the development of this EBV-associated cancer. We found that expression of EBNA1 in either EBV-negative (CNE2Z or HK1) or EBV-positive (C666-1) NPC cells led to loss of PML nuclear bodies and PML protein and that silencing of EBNA1 in C666-1 restored these levels. EBNA1 was observed by immunoflouresence microscopy to associate with the PML bodies in NPC cells and to co-immunoprecipitate with a single isoform of the PML protein suggesting a direct role in modulating PML. Mutational analyses of EBNA1 indicated that USP7 binding was required for PML disruption by EBNA1 and, in keeping with this result, USP7 silencing abrogated the ability of EBNA1 to disrupt PML bodies. PML bodies are known to be required for p53 activation (by acetylation), DNA repair and apoptosis, and therefore we examined the effect of EBNA1 on these processes. As expected, EBNA1 expression was found to inhibit all of these processes resulting in increased survival of cells with DNA damage after etoposide or UV treatment. These results suggest that EBNA1 contributes to the development of NPC through the disruption of PML nuclear bodies.

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EPSTEIN-BARR VIRUS (EBV) AND NASOPHARYNGEAL CARCINOMA (NPC)

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Abstract:

Infection with Epstein-Barr virus (EBV) is considered as the major contributor for Nasopharyngeal carcinoma (NPC), a disease with a remarkable geographic and ethnic distribution worldwide. We have previously reported genomic sequence of EBV GD1 strain from a NPC patient in Canton, one of the NPC endemic regions (Zeng MS et. al., JVI 2005). In this conference, we will discuss the new progresses made in our laboratory: 1) the profile of EBV strains in Cantonese based upon the sequence variations in LMP1 carboxyl-terminus, showing that China1 (Ch1) might be the susceptible strain for invading host in Canton; 2) 155849A in RPMS1 of EBV may represent a specific EBV subtype in the NPC endemic region, and could serve as a valuable indicator for a high risk of NPC in southern China. 3) IgA- and IgG-gp78 are novel biomarkers for NPC diagnosis by screening EBV serological parameters based on xMAP technology.

Key words: Epstein-Barr virus, nasopharyngeal carcinoma, LMP1; China 1, RPMS1, gp78, xMAP technology

4 (RegID: 1839)
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EBV AND HODGKIN'S LYMPHOMA: NEW INSIGHTS INTO AN OLD ASSOCIATION

Paul Murray, Martina Vockerodt, Karl Baumforth, Jennifer Anderton, Katerina Vrzalikova, Sarah Leonard, Zumla Cader, Shikha Bose, Ciaran Woodman and Lawrence Young
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Abstract:
A proportion of Hodgkin's lymphoma (HL) tissues harbour EBV within the malignant Hodgkin's/Reed-Sternberg (HRS) cells. Surprisingly, there are few differences between EBV-positive and EBV-negative HRS cells; emerging evidence suggests that while EBV is able to subvert cellular processes in favour of growth and survival, other cellular events may be necessary when EBV is absent. The challenge is to unravel this complexity by detailed consideration of the function of individual latent EBV genes in the appropriate cellular context. With this in mind, we have developed a method for the delivery of viral genes to primary human germinal centre B cells, the presumed progenitor of HL. Studies of the effects of EBV latent genes in primary B cells have until now been restricted to naïve B cells or to isolated GC B cells that have been immortalized with EBV. However, these models provide little information on the changes induced by individual latent genes relevant to the early stages of B cell transformation because the EBV-driven immortalization process is already complete. Our studies of the effects of EBV latent genes in primary B cells have so far provided new insights into the contribution of LMP1 to altered B cell differentiation, and in particular to the loss of B cell identity that is characteristic of HL. Our studies have also revealed that EBNA1 has hitherto unrecognized functions in HL cells, which include its ability to influence TGF-β signaling, while at the same time contributing to the recruitment and modification of T cells. Furthermore, we have shown that several EBV latent genes influence the activity of bioactive lipids such as sphingosine-1-phosphate (S1P). Not only have does S1P influence the phenotype of HL cells, but our preliminary evidence suggests that blocking its activity may be of therapeutic benefit in HL. The identification of novel cellular pathways targeted by EBV during the pathogenesis of HL can lead to the development of alternative therapies for this disease.

5 (RegID: 1840)
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EPTEIN-BARR VIRUS-INDUCED ONCOGENESIS THROUGH UTILIZING THE INNATE IMMUNITY SYSTEM

Kenzo Takada, Dai Iwakiri and Mrinal Samanta
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Abstract:
EBER is non-polyadenylated, untranslated RNA with a 170 nucleotides long. EBER is the most abundant viral RNA in latently EBV-infected cells, and is expected to form a double-stranded RNA (dsRNA)-like structure with many short stem-loops. Our series of studies have demonstrated that EBER plays key roles in oncogenesis. EBER confers resistance to apoptosis, induces expression of cellular growth factors, i.e. IL-10 in Burkitt's lymphoma, IL-9 in T/NK cell lymphoma and IGF-1 in gastric carcinoma and NPC, each of which acts as an autocrine growth factor, and plays a critical role in EBV-induced B-cell transformation.
As to the mechanism of EBER's action, we have found that EBER is recognized by retinoic acid-inducible gene I (RIG-I) and toll-like receptor 3 (TLR3), which are sensors of viral dsRNA in the innate immunity system, and activates downstream signaling to induce protective cellular genes, including type I interferon (IFN) and inflammatory cytokines. It was also demonstrated that EBER induces IL-10 and IGF-1 through the same signals. Although NF-κB is reported to function downstream of RIG-I signaling to induce inflammatory cytokines, our results have demonstrated that IFN-regulatory factor 3 (IRF-3) but not NF-κB is involved in the induction of an anti-inflammatory cytokine IL-10. We have also found that a substantial amount of EBER is detected in culture supernatants of EBV-infected cells and in sera of patients with active EBV infection, and EBER released from the EBV-infected cells induces signaling from TLR3, suggesting that the circulating EBER in patients with EBV-associated malignancies would play a role in oncogenesis.
These findings demonstrate a novel mechanism of oncogenesis utilizing the innate immunity system.

6 (RegID: 1947)
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THE MOLECULAR BIOLOGY OF EBV: FROM B95 TO NPC

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The University of North Carolina
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Abstract:
The Epstein-Barr virus (EBV) is a major human tumor virus that was discovered in Burkitt Lymphoma. Early biologic and molecular studies were dependent on cell lines that were established from BL or B-cell lines produced by infection in vitro. Two cell lines had unique properties. The B95 cell line was selected as a high virus producer while the HR-1 cell line was a non-transforming variant. Early studies compared the genomes initially using hybridization kinetics and restriction enzyme mapping. These studies indicated that each strain had unique deletions. The HR1 deletion was later shown to encompass the coding sequences for EBNA2 and the B95 deletion spans the BART transcripts and miRNAs.
EBV was also shown to be closely linked to the epithelial tumor, nasopharyngeal carcinoma, however. NPC cell lines were not available. Therefore the early molecular studies of EBV in NPC used primary NPC biopsy tissue. Many important aspects of EBV molecular biology have been discovered from the studies of NPC. These include the development of the EBV termini assay, a new pattern of viral expression with transcription of LMP1 and LMP2, identification of the TR LMP1 promoter, cloning of the BART transcripts, characterization of strain variation within LMP1, activation of the PI3kinase/Akt pathway by LMP2, and identification of multiple NFkB pathways. The significance of these findings from NPC will be presented.

NOTES:

Oral speaker
Abstract

Session 1: LATENT INFECTION (Ⅰ)

Chairpersons: Alan Rickinson, George Sai-Wah Tsao

Saturday, November 8, 08:55-10:15

7 (RegID: 1268; 1269）

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EPISOME STABILITY OF EBV IS REGULATED BY AN S-PHASE CHECKPOINT THAT DELAYS REPLICATION TIMING

Jing Zhou1, Andy Snyder1, and Paul M. Lieberman1,3

Oralabstract:

Epstein-Barr virus (EBV) episomes can be selectively eliminated from host cells by treatment with hydroxyurea (HU). We show here that HU treatment abrogates a checkpoint-mediated delay in the replication timing of EBV episomes. HU treatment causes hyperacetylation of histone H3 and a decrease in telomere repeat factor 2 (TRF2) binding at the EBV origin of plasmid replication (OriP). Deletion of TRF2 binding sites within OriP or siRNA depletion of TRF2 advanced the replication timing of OriP-containing plasmids. TRF2 forms a stable complex with HDACs1 and 2, and HU treatment was found to inhibit HDAC activity. TRF2 also formed a complex with the intra-S phase checkpoint protein Chk2. Chk2 could bind and phosphorylate TRF2 in vitro, and depletion of Chk2 phenocopies HU treatment. These findings suggest that a TRF2-HDAC-Chk2 complex delays replication timing at OriP through TRF2 phosphorylation and histone deacetylation at OriP. We propose that the TRF2-HDAC-Chk2 complex enhances EBV episome stability by providing an S-phase checkpoint that delays replication initiation at OriP.

8 (RegID:1079; 1080; 1081）
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CELL GENE REGULATION ASSOCIATED WITH EBER EXPRESSION

Goran Gregorovic, Daniel Charnock, Oliver Dittrich-Breiholz, Michael Kracht, Rainer Russ and Paul J. Farrell

Oralabstract：
Microarray gene expression profiling has been used to investigate cell genes whose expression correlates with expression of the Epstein-Barr virus EBER RNAs. New EBV mutants with deletions of EBER1 or EBER2 were constructed using BAC mutagenesis in E. Coli. The EBV-BACs were transferred to 293 cells and multiple cell lines containing wild type B95-8 EBV BAC or the EBER1 or EBER2 deletion mutants were cloned and characterised. RNA from 3 or 4 replica cell lines of each was used in microarray gene expression profiling. Bioinformatic analysis of the data showed that the patterns of cell gene expression of the wt and mutant virus lines could be distinguished by cluster analysis. Small numbers of genes reproducibly differed in expression by greater than 5 fold in the different groups of cell lines.

EBV lytic replication was induced in the 293 cell lines and the resulting virus (which carries a gfp marker) was titred in Raji cells, counting gfp positive cells. Equal titres of the virus stocks were used to infect primary human B cells isolated from peripheral blood or tonsils and lymphoblastoid cell lines (LCLs) were readily obtained from the EBV mutants and wt virus. No substantial difference in the efficiency of production of LCLs between the EBER mutants or wt BAC virus was observed in the conditions used. RNA from the LCLs was used for further microarray gene expression profiling and the results were compared to the 293 cell data. A panel of 30 genes selected from the microarray analysis was tested for expression by RT-PCR and the differential expression of RNA according to EBER expression in the LCL or 293 cell lines was confirmed. The results will be discussed in relation to potential roles for the EBER RNAs in EBV infection.**6**

9 (RegID: 1538)

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THE REGULATION OF EPSTEIN-BARR VIRUS LATENT GENE EXPRESSION BY CYTOKINES

Loránd L. Kis, Daniel Salamon, Emma Persson, Noémi Nagy, George Klein, and Eva Klein

Oralabstract:

The mechanisms that determine the different EBV latent viral gene expression programs seen in EBV-carrying normal and malignant cells are not known. In our studies we have focused on the regulation of LMP-1 expression in type II EBV latency.

In an in vitro model of EBV-positive HL, we found that the cytokines IL-4 and IL-13 could induce the expression of LMP-1 in the absence of EBNA-2. The molecular mechanism of the LMP-1 induction by IL-4 and IL-13 involved a direct signaling pathway with STAT6 as the signal transducer and a newly defined high-affinity STAT6-binding site in the LMP-1 promoter. Since 70-80% of the HL cases have the STAT6 signaling pathway activated, this signaling pathway may be involved in the expression of LMP-1 in the EBV-positive HLs.

Furthermore, we have identified IL-21 as a new cytokine that could induce LMP-1 in the absence of EBNA-2 in EBV-carrying, type I BL lines and in the estrogen-starved, conditional LCL ER/EB2-5. Interestingly, when LCLs were treated with IL-21 the cells differentiated to Ig-producing plasma cells with the concomitant down-regulation of Cp-driven mRNAs, but maintenance of LMP-1 expression. IL-21 is produced by CD4-positive follicular helper T cells in the germinal centers of secondary lymphoid follicles and CD4-positive T cells in HLs, and therefore it is possibly involved in the LMP-1 expression at these sites.

Altogether our results provide strong evidence for the involvement of extracellular signals in the regulation of latent viral gene expression in EBV-carrying cells, with potential therapeutic implications.

10 (RegID: 1448)

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EXPERIMENTAL RHESUS MACAQUE INFECTION WITH A RHESUS LYMPHOCRYPTOVIRUS MOLECULAR CLONE.

Makoto Ohashi, Angela Carville, Carol Quink and Fred Wang

Oralabstract:

Background: Natural and experimental infection of rhesus macaques with the EBV-related rhesus lymphocryptovirus (rhLCV) reproduces the biology of EBV infection in humans. We established a Bacterial Artificial Chromosome derived rhLCV clone in order to develop an experimental genetic system for this animal model of EBV infection.

Methods: The rhLCV molecular clone 16.5 was derived by homologous recombination into the viral episome genome, rescue in E. coli, replication in eukaryotic cells, and recovery of transforming virus by infection of rhesus PBMC.

Results: Clone 16.5 rhLCV was competent for viral replication and B cell immortalization in tissue culture. Southern blot and PCR analysis confirmed the presence of a signature lox-scar sequence at the site of homologous recombination in the rhBARF1 ORF. A SHIV-immunosuppressed, rhLCV-naïve macaque was orally inoculated to test whether the rhLCV clone 16.5 was capable of successfully infecting and persisting in vivo. Acute viral infection of the peripheral blood was detected by EBERs RT-PCR at day 21, and recovery of a spontaneous LCL from the peripheral blood confirmed the signature scar sequence for the rhLCV molecular clone. Persistent virus infection was established, and oral shedding of virus was detected.

Conclusion: We have established the first rhLCV molecular clone and infection of a rhesus macaque with a genetically engineered rhLCV. The rhLCV clone 16.5 is capable of acutely invading the peripheral blood after oral inoculation, establishing persistent infection in the peripheral blood, and reactivating for oral viral shedding. The results suggest that BARF1 is not essential for acute, persistent, or reactivated infection in an immunosuppressed host. These studies establish a new genetic system for studying the role of viral genes for LCV infection of the natural host.

NOTES:

Oral speaker
Abstract

Session 2: **LATENT INFECTION (Ⅱ)**

Chairpersons: **Friedrich Grasser, Kenzo Takada**

Saturday, November 8, 11:10-12:30

11 (RegID:1801)

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ESTABLISHMENT OF STABLE INFECTIN OF EPSTEIN-BARR VIRUS IN PREMALIGNANT NASOPHARYNGEAL EPITHELIAL CELLS.

1CM Tsang, 2E. Seto, 2K Takada, 3Y Cao, 4Chen HL, 5KW Lo, 6DY Jin, 7DLW Kwong, 1GT Zhang, 1YL Yip, 1PM Hau, 1C Man, 1LL Sheung, 1*SW Tsao.
1Dept. of Anatomy, 4Dept. of Microbiology, 6 Dept. of Biochemistry, 7Dept. of Clinical Oncology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, SAR China
5Dept. of Anatomical and Cellular Pathology, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong, SAR China
2Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan
6Cancer Research Institute, Xiangya School of Medicine, Central South University, Changsha 410078, Hunan, China

Oralabstract:

Epstein Barr virus (EBV) infection is closely associated with nasopharyngeal carcinoma (NPC) and has been postulated as an etiological factor for the development of nasopharyngeal carcinoma. EBV infection was shown to be an early event in the pathogenesis of NPC. The lack of representative premalignant nasopharyngeal epithelial cell system for EBV infection has hampered research to elucidate events regulating EBV infection and the role of EBV infection in NPC pathogenesis. Recently, we have established and characterized a telomerase-immortalized nasopharyngeal epithelial cell, NP460hTert. Here, we reported the successful establishment of stable EBV infection in this premalignant nasopharyngeal epithelial cell system upon long term propagation NP460hTert-EBV expressed latent genes of EBV including EBER, EBNA1 and LMP1. The EBV infected cells were also responsive to lytic induction by TPA, with the expression of BZLF1 and BMRF1 being effectively induced. However, the lytic infection of EBV infection in nasopharyngeal epithelial cells is abortive in nature as no infectious EBV particles could be detected in the culture supernatant. The EBV infected premalignant nasopharyngeal epithelial cells exhibited some transformed properties including invasive growth property in 3-dimensional collagen gel and anchorage independent growth in soft agar. STAT3 was activated in the EBV-infected nasopharyngeal epithelial cells together with upregulated cytokines which may support cell growth and survival. The EBV-infected nasopharyngeal epithelial cells remain non-tumorigenic in athymic nude mice indicating additional events are required to complete the malignant transformation of these cells.

12 (RegID:1729)

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BZLF1 PROMOTER SILENCING ELEMENTS ZV/ZV' AND ZIIR FUNCTION SYNERGISTICALLY IN ESTABLISHING AND MAINTAINING EBV LATENCY

Xianming Yu, Patrick J. McCarthy, Richard J. Kraus, Amy Ellis, Zhenxun Wang, Hui Jun Lim, and Janet E. Mertz.

Oralabstract:

The EBV BZLF1 gene encodes a protein, Zta, which plays a central role in regulating the switch between latency and lytic replication. Liu et al. (J. Virol. 72:8230, 1998) previously identified a silencing element in the BZLF1 promoter, Zp, named ZIIR. We identified another Zp silencing element, ZV, which binds the cellular factors ZEB1 and ZEB2/SIP1 (Kraus et al., J. Virol. 77:199, 2003). Recently, we reported that a 2-bp substitution mutation in ZV in the context of a whole genome of EBV strain B95.8 (Yu et al., PLoS Pathogens 3(12) e194, 2007) leads to spontaneous reactivation out of latency with production of infectious EBV in epithelial 293 cells under conditions in which WT EBV never does. Human B-lymphocytic BJAB cells latently infected with this mutant also accumulated at least 5- to 10-fold more Zta mRNA than did WT-infected ones. We also identified a second, synergistically acting ZEB1/2-binding site in Zp, named ZV'.

We have now constructed both a Zp-driven reporter and whole EBV genomes containing various combinations of mutations in these silencing elements. Mutation of ZIIR was found to relieve repression of Zp in a cell type-dependent manner. 293 cells infected with these mutant viruses spontaneously reactivated out of latency, with frequency and virus production ZV/ZV'/ZIIR triple mutant > ZIIR mutant > ZV/ZV' double mutant > ZV mutant >WT. Importantly, all of these mutant viruses are at least 10-fold defective in inducing proliferating colonies following infection of primary human B cells, with the ZV/ZV'/ZIIR triple mutant exhibiting the greatest defect. We conclude that Zp contains two or more silencing elements in addition to MEF2D-binding sites which synergize in establishing and tightly maintaining EBV latency in a cell-type dependent manner. Studies in progress include identifying the ZIIR-binding factor(s) and determining the functions of this repressor and ZEB1/2 in establishing EBV latency.

13 (RegID: 1742)

Natalie Sutkowski
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INTERACTION OF EXOGENOUS AND ENDOGENOUS VIRUSES IN HEAD AND NECK CARCINOMA

Semyon Rubinchik, Jacob Smith, Brian Hoel, Lucinda Halstead, David White, Marion B. Gillespie, and Natalie Sutkowski

Oralabstract:

Oncogenic viruses are etiologic agents in two forms of head and neck cancer. Epstein-Barr virus (EBV) is associated with virtually all undifferentiated nasopharyngeal carcinomas (NPC), and human papillomavirus (HPV) is associated with approximately half of oropharyngeal squamous cell carcinomas (OSCC). We previously discovered that EBV transactivates a human endogenous retrovirus, HERV-K18, that encodes a superantigen in its envelope gene. Superantigens cause strong T cell activation and cytokine production, resulting in inflammation. Recently, we found that HPV also induces this superantigen in epithelial cells, suggesting a possible etiological role for HERV-K18 related inflammation in virally associated head and neck cancers. In support of our findings, superantigen transcripts were detected in 20/20 EBV+ NPC specimens tested, and were significantly increased In HPV+ OSCC compared with HPV- tumors. HPV16 E6 and E7 oncoproteins were each sufficient to transactivate the HERV-K18 superantigen in primary tonsil (oropharyngeal) epithelial cells, while EBV LMP2 was sufficient to transactivate the superantigen in primary adenoid (nasopharyngeal) epithelial cells. We hypothesize that HERV-K18 superantigen activated T cells affect virally associated head and neck cancers by eliciting a localized inflammatory response that could either promote or inhibit carcinogenesis, depending upon the T cells present in the tumor microenvironment. Superantigen associated T cell proliferation might result in expansion of either effector or regulatory responses, while activation induced cell death might cause functional tolerance of either T cell subset, altering the balance between cytotoxicity and T cell suppression.

14 (RegID: 1153; 1154)

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METHYLATION GENERATES FUNCTIONALLY DIFFERENT SUBSPECIES OF THE EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 2

Henrik Gross, Alfredo Mamiani, Stephanie Barth, Christine Hennard, Ursula-Zimber-Strobl, Elisabeth Kremmer, and Friedrich A. Grasser

Oralabstract:

The Epstein-Barr virus-encoded nuclear antigen 2 (EBNA2) is essential for the transformation of B-lymphocytes by EBV. The symmetrically dimethylated Arginine (sDMA) residues within its Arginine-Glycine (RG) repeat confer binding to the survival of motor neurons protein (SMN). With newly developed methylation-specific monoclonal antibodies, we now show that the RG repeat also contains non-methylated (NMA) as well as asymmetrically dimethylated Arginines (aDMA). Non-methylated EBNA2 sediments in a low molecular weight fraction, while the majority of aDMA- and sDMA-modified EBNA2 migrates to high-molecular mass fractions in a sucrose gradient. The methylation status of the EBNA2-subfractions did not change upon lytic viral replication but was substantially reduced by the methylation inhibitor AdOx. Non-methylated EBNA2 did not bind to DNA in an electrophoretic mobility shift assay (EMSA) but could be transformed in vitro to a methylated, DNA-binding form that was supershifted by the aDMA-specific antibody while the sDMA-specific antibody did not induce a supershift. Our data indicate that methylation of EBNA2 generates functionally divergent subspecies and that methylation is a prerequisite for DNA-binding by EBNA2 via association with the transcription factor RBPJκ.

NOTES:

Oral speaker
Abstract

Session 3: **VIRAL REPLICATION**

Chairpersons: **Alison Sinclair, Paul Farrell**

Saturday, November 8, 13:40-15:20

15 (RegID:1042; 1043; 1044)

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THE EPSTEIN-BARR VIRAL PROTEIN EB2 STRONGLY STIMULATES TRANSLATION OF INTRONLESS RNAS INDEPENDENTLY OF PKR

Emiliano Ricci, Fabrice Mure, Henri Gruffat, Didier Décimo, Cahora Medina-Palazon, Théophile Ohlmann and Evelyne Manet

Oralabstract：

The Epstein-Barr Virus (EBV) early nuclear protein, EB2 (also called Mta, SM or BMLF1), allows the nuclear export of a certain subset of early and late viral RNAs derived from intronless genes and its presence is essential for the production of infectious particles. With the growing evidence that cellular mRNA splicing and export factors are able to regulate translation of spliced mRNAs it was of interest to test whether EB2 could also have an effect on translation.

By using an intronless luciferase coding construct and quantitative RT-PCR from cytoplasmic RNAs we show that, beside its role as a nuclear export factor, EB2 strongly stimulates expression of unspliced RNAs. This stimulation occurs at the translational level and does not depend on any specific RNA sequence as demonstrated by its ability to stimulate both translation of EBV derived RNAs and non related reporter genes. In fact, EB2 specifically enhances translation of intronless RNAs without significantly affecting overall expression of cellular mRNAs as showed by metabolic labeling of cells expressing EB2. This was confirmed by the addition of an intron within the 5'UTR of the luciferase coding construct which led to a lack of effect of EB2 on its translation. Moreover, mRNA intake by EB2 has to occur within the cell nucleus, as translation of RNAs transfected directly into the cytoplasm of EB2 expressing cells is not stimulated. We also show that the mechanism of translation stimulation driven by EB2 is independent of PKR, the main factor involved in the interferon-mediated inhibition of viral translation.

Finally, the test of EB2 homologs from EBV related viruses (HSV-1, CMV, KSHV) lead to the conclusion that even if they play similar role within the replication cycle, their effect on translation is not conserved between all of them.

16 (RegID: 1989)
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MURINE GAMMAHERPESVIRUS-68 ORF33 ENCODES A TEGUMENT PROTEIN ESSENTIAL FOR VIRION EGRESS

Haitao Guo1 and Hongyu Deng1,2
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Oralabstract：
The tegument which surrounds the nucleocapsid and interacts with the virion envelope plays important roles in herpesvirus life cycle, although the function of individual tegument proteins has not been well understood. Murine gammaherpesvirus 68 (MHV-68) is genetically related to two human gammaherpesviruses, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8), which are associated with several types of human malignancies. De novo infection of MHV-68 in cell cultures is efficient and yields high viral titers, providing a robust system to study the structure and assembly of gammaherpesviruses. MHV-68 open reading frame 33 (ORF33) is conserved among all herpesvirus subfamilies, but its specific role in viral lytic replication has not yet been determined. We describe here that ORF33 is a true late gene and encodes a tegument protein. The green fluorescent protein fusions to ORF33 (GFP-33) were distributed evenly in the cytoplasm and nucleus, and GFP-33 accumulated in nuclear compartments at late phase of viral infection. To study the role of ORF33 in MHV-68 lytic replication, we constructed an ORF33-null MHV-68 mutant (33STOP) by insertion of triple translation termination codons into the N terminus of ORF33 in MHV-68 genome on a bacterial artificial chromosome. We demonstrated that ORF33 was not required for viral DNA replication, immediate early, early and late gene expression, viral DNA packaging or nucleocapsid assembly. However, the 33STOP mutant was defective in the assembly and release of mature virion particles, as revealed by electron microscopic studies. Our results indicate that ORF33 is essential for MHV-68 lytic replication and functions in the tegumentation and egress of mature viral particles in the cytoplasm.

17 (RegID: 1773)
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HIGH EXPRESSION LEVELS OF STAT-3 ARE CHARACTERISTIC OF CELLS REFRACTORY TO EPSTEIN-BARR VIRUS LYTIC CYCLE INDUCTION

Derek Daigle, Cynthia Megyola , Ayman El-Guindy, Lyn Gradoville, George Miller, Sumita Bhaduri-McIntosh - Yale University

Oralabstract:

More than 50% of cells in Burkitt lymphoma lines latently infected with Epstein-Barr virus (EBV) are refractory to viral lytic cycle induction in vitro. What is responsible for the refractory state is unclear. Using a method that we have described, we separated lytic from refractory cells following treatment with an HDAC-inhibitor, sodium-butyrate (Na-B). When refractory cells were re-introduced into culture, cells were initially refractory to lytic cycle inducing agents of different classes. However, refractoriness to lytic cycle induction diminished with time to levels equivalent to those observed in parental, un-separated cells. Thus, latently infected cells are not permanently marked for refractoriness to lytic cycle induction. There was increase in acetylation of histone 3 globally and in the region of the BZLF1 promoter in both refractory and lytic populations following treatment with Na-B as compared to untreated cells. This indicates that refractoriness was not a result of lack of response to NaB. We compared levels of expression of host cell genes in untreated- and Na-B-treated cells. Experiments using Affymetrix U133 Plus 2.0 human genome arrays revealed differential patterns of expression of genes with potential roles in EBV oncogenesis. Using qRT-PCR, we confirmed that expression levels of Stat-3 and c-Fos were increased in Na-B-treated as compared to untreated cells. Furthermore, considerably higher levels of expression of Stat-3 and c-Fos were detected in refractory than in untreated or lytic cells by microarray and qRT-PCR using separated cells. Increase in STAT-3 protein levels in refractory cells was confirmed by Western blotting. Flow cytometry revealed that while the level of STAT-3 protein was increased in all cells treated with Na-B, very high levels of STAT-3 were observed only in refractory cells. Thus, STAT-3 is closely tied to refractoriness of cells to lytic cycle induction.

18 (RegID: 1263)
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GENE SPECIFIC ACTIVATION AND RNA BINDING BY EBV SM PROTEIN

Zhao Han, Dirk Dittmer and Sankar Swaminathan

Oralabstract:
EBV SM protein (also known as Mta, BMLF1, EB2) is a posttranscriptional regulatory protein expressed early during lytic replication that is essential for virus production. SM is an RNA binding protein that enhances accumulation of its target mRNAs by multiple mechanisms including effects on RNA stability, processing and export. We had previously shown that SM preferentially enhances accumulation of EBV transcripts, and that approximately 40% of EBV mRNAs are SM-dependent. It is not known whether preferential gene activation by SM is due to transcript-specific differences in the ability to associate with SM protein or due to intrinsic differences in stability or exportability of various mRNAs. Understanding the basis of gene specific activation by SM should provide insights into the regulation of lytic EBV replication and opportunities for specific therapeutic interventions. To ask whether SM associated more efficiently with specific EBV mRNAs, we employed an RNA immunoprecipitation/ quantitative RT-PCR assay. Cell lines derived from lymphomas infected with EBV were genetically modified to permit inducible high level lytic EBV replication were used as a source of EBV mRNAs. Cells were induced to permit lytic replication, lysed and SM/RNA complexes were immunoprecipitated with SM-specific antibody or pre-immune serum. RNA was isolated from each immunoprecipitation and analyzed by RT-PCR microarray for all EBV ORFs. We found that there is a general enrichment of EBV RNA in SM-immunoprecipitates, indicating that SM has non-specific RNA binding capability. However, there were several transcripts which were highly enriched in the SM immunoprecipitates, suggesting that SM binds preferentially to specific RNAs. In order to map potential high affinity SM-binding sites, protein-RNA crosslinking assays were employed to compare the affinity of SM for various portions of these EBV mRNAs. These assays utilized constructs encoding different regions of SM-associated mRNAs from the 5'UTR to the cleavage and polyadenylation site to generate radiolabeled transcripts. The results of these
assays will be discussed.

19 (RegID:1252; 1254; 1255）

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EPSTEIN-BARR VIRUS COLONISATION OF B CELL SUBSETS IN PRIMARY INFECTION AND PERSISTENCE

S. Chaganti, A. Bell, C. Ma, D. Croom-Carter, E. Heath, S. Tangye, A. Hislop, W. Bergler, M. Kuo, M. Buettner, G. Niedobitek and A.B. Rickinson

Oralabstract:

The human circulating B cell pool is composed of three populations: (i) naïve (IgD+ CD27-) cells that have not yet engaged antigen, (ii) isotype-switched (IgD- CD27+) memory cells that arise through antigen stimulation and germinal centre (GC) transit and (iii) non-switched (IgD+ CD27+) memory cells whose origin remains unclear but recent evidence suggests may arise in a GC-independent manner. EBV persists in the immune competent host by preferentially colonising class-switched memory B cells, although how this is achieved remains unclear. In one scenario, EBV infects naïve cells and drives their differentiation into memory via GC transit; in another, EBV avoids GCs and infects memory cells directly. Here we report three findings which support this latter view. Firstly we analysed virus genome loads in isolated tonsillar B cell subsets from infectious mononucleosis (IM) patients and found that EBV loads were relatively low in CD10+ or CD77+ GC cells but were concentrated in extrafollicular B cells expressing the GC marker CD38 as an activation antigen. Secondly we examined EBV's colonisation of circulating naïve, class-switched and non-switched memory B cells and found that, while switched-memory B cells had the highest viral loads, EBV was also detectable in non-switched memory cells both in IM patients and in some long-term virus carriers. Thirdly, we investigated EBV carriage in patients with X-linked lymphoproliferative disease (XLP), an immunodeficiency characterised by extreme susceptibility to EBV infection, the absence of fully-functional GC activity and the inability to generate class-switched memory B cells. Here we found that XLP patients who survived primary infection harboured EBV within the small population of non-switched memory B cells, rather than within the dominant naïve population. These findings suggest that EBV can colonise the non-switched memory B cell pool, albeit not as efficiently as it does the switched memory population, and that EBV can establish persistence in the absence of GC transit.

NOTES:

Oral speaker
Abstract

Session 4: GENETICS, NON-CODING RNAS AND EPIGENETIC

Chairpersons: Paul Ling, Ingemar Ernberg

Saturday, November 8, 15:40-17:20

20 (RegID: 1132)

Qian Tao
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EPIGENETIC ETIOLOGY OF NASOPHARYNGEAL CARCINOMA

Qian Tao

Oralabstract:

Carcinogenesis is a multi-step process, involving multiple genetic and epigenetic alterations including the epigenetic disruption of tumor suppressor genes (TSGs) through promoter CpG methylation. The frequent presence of epigenetic abnormalities in tumor cells provides us with potential epigenetic biomarkers for molecular cancer diagnosis and also a novel way of identifying new TSGs. Using DNA methyltransferase inhibitors as demethylation agents, epigenetic inactivation of TSGs can also be reversed and exploited as a cancer therapeutic strategy. Using nasopharyngeal carcinoma (NPC) as a tumor model which is prevalent in southern China, I attempted to identify epigenetically inactivated novel TSGs, by employing integrative epigenetic/genomic approaches such as methylation subtraction, array-CGH, high-throughput expression profiling coupled with methylation analysis, and functional studies. A few novel candidate TSGs have been identified, including cell signaling-related and transcriptional regulatory genes. These genes are frequently methylated and downregulated in common tumors including NPC. Functional analyses showed that these candidate genes could induce apoptosis of tumor cells and suppress tumor cell growth, are thus functional TSGs. The epigenetic silencing of these TSGs would disrupt normal cell signaling control and contribute to NPC pathogenesis.

21 (RegID: 1125)
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MIR-BART5 IS AN ANTI-APOPTOTIC MIRNA ENCODED BY EBV AND INHIBITS PUMA TO PROMOTE HOST CELL SURVIVAL

Elizabeth Yee-Wai Choy, Ka-Fai To, Dora Lai-Wan Kwong, Sai Wah Tsao and Dong-Yan Jin

Oralabstract:

Epstein-Barr virus (EBV) is a herpesvirus associated with nasopharyngeal carcinoma (NPC) and other types of epithelial and lymphoid malignancies. EBV is the first human virus found to express microRNAs (miRNAs), but the functions of these viral miRNAs are largely unknown. Here, we identified and characterized a cellular target of an EBV miRNA known as miR-BART5, which is conserved with rhesus lymphocryptovirus miR-rL1-8 and abundantly expressed in NPC cells. By in silico analysis and functional screening with luciferase reporter assays, we showed that miR-BART5 targets the 3-untranslated region of a cellular mRNA transcript encoding p53 upregulated modulator of apoptosis (PUMA), a mediator of p53-dependent and –independent apoptosis. The target site of miR-BART5 was identified and characterized. Regulation of the endogenous PUMA expression was verified by overexpressing miR-BART5. In addition, a synthetic anti-miR-BART5 oligonucleotide inhibitor was used to confirm that reduction of endogenous miR-BART5 in NPC cells led to alteration in PUMA expression. Consistent with a role in the development of NPC, PUMA was found to be significantly underexpressed in about 60% of human NPC tissues constitutively harboring EBV. More importantly, miR-BART5-expressing NPC cells were less sensitive to proapoptotic agents adriamycin and etoposide. In addition, apoptosis can be induced in these cells by inhibiting miR-BART5 activity. Taken together, our findings suggest a model for viral promotion of tumor cell survival in which EBV encodes an miRNA to repress the expression of PUNA and consequently facilitate the establishment of stable latent infection and the development of epithelial carcinoma.

22 (RegID: 1165)
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EBV LATENCY IN B CELLS LEADS TO EPIGENETIC REPRESSION AND CpG METHYLATION OF THE TUMOUR-SUPPRESSOR GENE BIM

Kostas Paschos, Paul Smith, Emma Anderton, Jaap M Middeldorp, Robert E White and Martin J Allday

Oralabstract:

In human B cells infected with EBV, latency-associated viral gene products inhibit expression of the pro-apoptotic Bcl-2-family member Bim and enhance cell survival. This involves the activities of the nuclear oncoproteins EBNA3A and EBNA3C and appears to be predominantly directed at regulating Bim mRNA levels, although post-translational regulation of Bim has been reported. The level of Bim is a critical regulator of lymphocyte survival and reduced expression is a major contributor to lymphoproliferative disease in mice and humans. Moreover Bim regulation is uniquely important in the pathogenesis of Burkitt's lymphoma (BL), since in this cancer Myc is deregulated by gene translocation and can induce apoptosis via Bim.

Here we show that protein and RNA stability make little or no contribution to the EBV-associated repression of Bim levels in latently infected B cells. However, treatment of cells with inhibitors of histone deacetylase (HDAC) and DNA methyltransferase (DNMT) enzymes indicated that epigenetic mechanisms are involved in the down-regulation of Bim. This was initially confirmed by chromatin immunoprecipitation analysis of histone acetylation levels on the Bim promoter. Subsequently methylation-specific PCR (MSP) and bisulphite sequencing of regions within the unusually large CpG island located at the 5' end of Bim revealed significant methylation of CpG dinucleotides in all EBV-positive, but not EBV-negative B cells examined. Genomic DNA samples exhibiting methylation of the Bim promoter included recently explanted EBV-positive BL biopsies.

We conclude that latent EBV initiates a chain of events that leads to epigenetic repression of the tumour suppressor gene Bim in infected B cells and their progeny. This reprogramming of B cells could have important implications for EBV persistence and the pathogenesis of EBV-associated disease, in particular BL. Moreover, reprogramming by EBV may also play a role in the development of other chronic disorders such as autoimmune disease.

23 (RegID:1933; 1934; 1775）
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NOVEL TUMOUR SUPPRESSOR GENES ASSOCIATED WITH INITIATION AND PROGRESSION OF NASOPHARYNGEAL CARCINOMA

Ning Wang, Tanya Pavlova, Lin Mo, Veronika Zabarovska, Zhiming Du, Vladimir Kashuba, Guangwu Huang, Maria Lung, Ingemar Ernberg, Eugene Zabarovsky and Lifu Hu*

Oralabstract:
Silencing of tumor suppressor genes (TSG) or activation of oncogene is a main mechanism for carcinogenesis. It often coincides with the aberrant methylation of CpG dinucleotides in CpG islands, frequently located in gene promoters and transcription start sites and plays an important role in various fundamental pathways, such as apoptosis, DNA damage repair, tumor invasion and metastasis. These regions of high CpG content are mostly unmethylated in normal cells, but high methylated in cancer as a common mechanism for the inactivation of TSGs. CpG island methylation phenotype (CIMP) is also considered to be a characteristic feature for the tumorigenesis.
To identify novel tumor specific and methylation regulated genes, we used our established method of substrate hybridisation to NotI microarray since this method could provide the opportunity to detect simultaneously and differentially copy number and methylation changes. Thus, it allows checking cancer cells for genetic and epigenetic abnormalities. Several pairs of normal-tumor and EBV LMP1 positive-negative samples are applied. In the present study, 190 genes from human chromosome 3 were analyzed and six genes were found to associate with NPC and four genes are responsive to EBV LMP1. Among them, PBSP3, WNT7A and ITGA9 are methylated genes, confirmed by 5´aza C treated NPC cell line and expression. The role of these genes in initiation and progression of NPC is on study.
These results supported the hypothesis on simultaneous inactivation of cluster cancer-causing genes during the development and progression of NPC. It could be important for development of specific biomarker sets for early diagnosis and new therapeutic approaches for cancer treatment.

24 (RegID:1184; 1185; 1186）

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ABILITY OF ZTA TO INTERACT WITH METHYLATED ZRES IN HUMAN AND VIRAL PROMOTERS: ROLE IN OVERTURNING EPIGENETIC SILENCING?

Questa H. Karlsson1, Celine Schelcher1, Elizabeth Verrall1, James Heather1, Kirsty Flower1, Carlo Petosa2 and Alison J. Sinclair1*

Oralabstract：

Zta (BZLF1, ZEBRA) is the key mediator of Epstein-Barr virus (EBV) lytic cycle. Zta interacts with specific sequence elements (ZREs) in both responsive promoters and the viral origin of lytic replication. Epigenetic control of gene expression is driven or reinforced by methylation of CpG motifs within regulatory regions. Zta has the unique property of enhanced interaction with methylated CpG-containing ZREs (as shown by Kenney's group).

Here we identify a CpG-containing ZRE in the human egr1 gene, which is both known to be regulated by Zta and required for EBV lytic replication. We map the relevant nucleotides within the ZRE and demonstrate that Zta interaction is strongly enhanced by methylation. Egr1 methylation is known to occur in B-cells and the ability of Zta to interact with methylated egr1-ZRE may aid its ability to activate egr1.

Furthermore, we demonstrate that Zta plays a role in overturning epigenetic silencing of the EBV genome. Activation of the BRLF1 promoter (Rp) by Zta is a key step in the activation of EBV lytic cycle. Rp contains three ZREs and two of these contain CpG motifs that are methylated in vivo. Through biochemical analyses and molecular modeling of Zta bound to RpZRE3, we identified the precise contacts made between a serine and a cysteine residue of Zta with the methyl cytosines. We identify a single point mutant of Zta, ZtaC189S that is specifically defective for binding to methylated RpZRE3 both in vitro and in vivo. This provided a tool to question the relevance of the interaction of Zta with methylated RpZRE3. We discovered that ZtaC189S was defective in activating Rp in Raji cells, which demonstrated the relevance of the interaction of Zta with a methylated ZRE.

This research establishes a role for Zta in overturning the epigenetic control of viral latency. The potential Zta to reprogram the host epigenome is under investigation.

NOTES:

Oral speaker
Abstract

Session 5: **SIGNAL TRANSDUCTION (Ⅰ)**

Chairpersons: **Lawrence Young, Martin Allday**

Sunday, November 9,08:25-10:25

25 (RegID:1109)

Zhenguo Wu
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MECHANISTIC STUDY OF THE LMP1-MEDIATED NON-CANONICAL NF-KAPPAB PATHWAY

Po-yan Cheung and Zhenguo Wu

Oralabstract：

Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) is oncogenic and indispensable for EBV-mediated B cell transformation. When LMP1 is expressed in host cells, it is capable of activating both canonical and non-canonical NF-kappaB pathways. We and others previously showed that the Carboxyl terminal activating region 2 (CTAR2) of LMP1 mainly engages the canonical NF-kappaB pathway by sequentially activating TRAF6, TAK1, and the IKK complex. In contrast, the CTAR1 domain of LMP1 was thought to mainly engage the non-canonical NF-kappaB pathway (refs1-4). However, less is known about the underlying mechanism. We show here that both CTAR1 and CTAR2 contribute to the activation of the non-canonical NF-kappaB pathway. TRAF3 binds to NIK and negatively regulates the non-canonical NF-kappaB pathway by promoting the degradation of NIK. Interestingly, LMP1 stabilizes NIK by promoting dissociation of TRAF3 from NIK. In addition, we find that TRAF2 is required for LMP1-induced p52 upregulation. Our findings provide more insights into the molecular mechanism underlying the LMP1-mediated non-canonical NF-kappaB pathway.

26 (RegID:1131)
Christophe LE CLORENNEC
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MOLECULAR BASIS OF CYTOTOXICITY OF EPSTEIN-BARR VIRUS (EBV) LATENT MEMBRANE PROTEIN 1 (LMP1) IN EBV LATENCY III B CELLS: LMP1 INDUCES TYPE II LIGAND-INDEPENDENT AUTOACTIVATION OF CD95/FAS WITH CASPASE 8-MEDIATED APOPTOSIS.

Christophe LE CLORENNEC Le Clorennec C (1), Ouk TS (2), Youlyouz-Marfak I (2), Panteix S (2), Martin CC, Rastelli J (3), Adriaenssens E (4), Zimber-Strobl U (3), Coll J (5), Feuillard J (2), Jayat-Vignoles C (2)

Oralabstract:
The Epstein-Barr virus (EBV) oncoprotein latent membrane protein 1 (LMP1) is thought to act as the major transforming protein in various cell types, by rerouting the tumor necrosis factor receptor family signaling pathway. Despite this implication in EBV-associated transformation of cells, LMP1 toxicity is a well-known but poorly studied feature, perhaps because it contradicts its role in transformation. We show that LMP1 physiological levels are very heterogeneous and that the highest levels of LMP1 correlate with FAS overexpression and spontaneous apoptosis in lymphoblastoid cell lines (LCLs). To understand the cytotoxic effect of LMP1 in LCLs, we cloned wild-type LMP1 into a doubly doxycycline-inducible episomal vector pRT-1, with a truncated version of NGFR as a surrogate marker of inducibility. We found that LMP1 overexpression induced apoptosis in LCL B cells, as shown by annexin V labelling, sub-G1 peak, and poly(ADP ribose) polymerase cleavage. Knocking down FAS expression by small interfering RNA abolished LMP1-induced apoptosis. The absence of detectable levels of FAS ligand mRNA suggested a ligand independent activation of FAS. LMP1 induced FAS overexpression with its relocalization in lipid raft micro-domains of the membrane. FAS immunoprecipitation detected FADD (FAS-Associated Death Domain protein) and caspase 8, suggesting a FAS-dependent formation of the death-inducing signalling complex. Caspases 8, 9, 3, and 7 were activated by LMP1. Caspase 8 activation was associated with BID cleavage and truncated-BID mitochondrial relocalization, consistent with type II apoptosis. Therefore, our results are in agreement with a model where LMP1-dependent NF-kappaB activation induces FAS overexpression and auto-activation that could overwhelm the anti-apoptotic effect of NF-kappaB, revealing an ambivalent function of LMP1 in cell survival and programmed cell death.

27 (RegID:1191; 1192）

Arnd Kieser
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PROTEOMIC IDENTIFICATION OF THE TYROSINE PHOSPHATASE SHP1 AS A NOVEL LMP1 INTERACTION PARTNER WHICH MEDIATES AUTOREGULATION OF LMP1 SIGNALING

Janine Griese 1, Thomas Kn?fel 1, Helmut Kutz 1, Stephan M. Feller 2, Arnd Kieser 1

Oralabstract:

LMP1 mimics a constitutively active receptor which activates NF-kappaB, JNK, MAPK, JAK/STAT and PI3K signaling. LMP1 recruits TRAFs, TRADD and RIP1 which are also known as signaling mediators of Toll-like and TNF-receptors. Here, we established a functional proteomics approach to identify novel interaction partners of the LMP1 signaling domain. This approach led to the characterization of the tyrosine phosphatase SHP1 as a direct binding partner of LMP1. Interaction of both molecules was verified in primary human B-cells which had been transformed with a recombinant EBV carrying an HA-tagged LMP1 allele. The SHP1 binding site of LMP1 is located within the membrane-proximal region of the LMP1 signaling domain and shows no overlap with known protein interaction domains of LMP1. The unique sequence of this site does not resemble known SHP1 interaction motifs of cellular proteins. Mutation of the SHP1 site caused the loss of SHP1 binding to LMP1 in lymphoblastoid cells. SHP1 has previously been described as a negative regulator of growth factor or immune receptor signaling by dephosphorylating e.g. tyrosine kinases such as JAKs or SRC kinases. LMP1 induction of NF-kappaB was greatly enhanced in SHP1-knockout DT40 B-cells as compared to wildtype cells. This effect was reverted by reconstitution of SHP1 expression in the SHP1-KO cells. Also mutation of the SHP1 interaction site or the co-expression of a dominant-negative SHP1 caused hyperactivation of NF-kappaB and JAK3 hyperphosphorylation by LMP1. Due to its inhibitory function and in analogy to the CTAR domains, we named the SHP1 binding region of LMP1 the C-terminal inhibitory region 1 (CTIR1). We described a novel autoregulatory mechanism of LMP1 signaling which limits its own activity through the recruitment of a tyrosine phosphatase. This mechanism might be of relevance for the survival of EBV-transformed cells because LMP1 hyperactivity is known to be toxic.

28 (RegID: 1012)
Xuechi Lin
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REGULATION OF MAPK MEDIATED OP18/STATHMIN SIGNALING PATHWAY TRIGGERED BY EBV ENCODED LMP1 IN NASOPHARYNGEAL CARCINOMA CELLS

Xuechi Lin, Sufang Liu, Lili Guo, Zijian Li, Xiaoqian Ma, Lili Li, Xiyun Deng, Ya Cao＃

Oralabstract：
Op18/stathmin is a molecule proposed to relay integrating diverse cell signaling pathways, the regulation of Op18/stahmin signaling can cause crucial changes of cellular biological properties including cell growth and proliferation. Our studies focused on the regulation of Op18/stathmin signaling by LMP1 in nasopharyngeal carcinoma (NPC) cells, Our results showed that LMP1 didn't affect the expression of Op18/stathmin , but LMP1 promoted the phosphorylation of Op18/stathmin. LMP1 was able to regulate Op18/stathmin signaling through MAPK mediation, but the activity of MAPK induced by LMP1 was not constant at different phases, changed with the cell cycle progression. LMP1 up-regulated the phosphorylation of MAPK at G1/S. On the contrary, LMP1 negatively regulated the phosphorylation of MAPK at G2/M phase. Inhibition of LMP1 expression attenuated the interaction of MAPK with Op18/stathmin and promoted microtubule depolymerization. All these revealed a new pathway through which LMP1 regulates Op18/stathmin signaling by MAPK mediation. The new signaling pathway on LMP1 which is closely associated with cell cycle progression not only perfects the LMP1 regulation network, but also provides new insights for elucidating the molecular mechanism of LMP1 leading to tumorigenesis.

29 (RegID:1213)
Yao Chang
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INDUCTION OF EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 BY ENDOPLASMIC RETICULUM STRESS IN NASOPHARYNGEAL CARCINOMA CELLS

Yao Chang, Jenn-Ren Hsiao, Chaio-Wei Chen, Shih-Yi Wu, Mei-Chi Hsu, and Ih-Jen Su

Oralabstract:

Endoplasmic reticulum (ER) stress occurs when functions and homeostasis of ER are disturbed. To reduce the stress, multiple events of cell signaling and gene regulation are activated, resulting in unfolded protein response (UPR). Notably, overexpression of UPR-related genes has been detected in several solid tumors, correlating with the malignant stages, metastasis and drug resistance. Previous studies have also indicated that ER stress-activated UPR can help tumor survival under hypoxia or treatment with chemotherapeutic drugs. It is unknown whether ER stress occurs in and contributes to nasopharyngeal carcinoma (NPC), an Epstein-Barr virus (EBV)-associated epithelial malignancy. In this study, we found that UPR markers are frequently detected in NPC biopsies and that ER stress inducers upregulate an EBV oncoprotein, latent membrane protein 1 (LMP1), which has been involved in malignant phenotypes of NPC through promoting cell growth, survival, invasive migration and angiogenesis. We further found that ER stress induces LMP1 at a transcriptional level and identified an ER stress responsive element in the distal promoter of LMP1 gene. Under ER stress, the UPR protein XBP-1s binds to the element and transactivates the LMP1 promoter. In addition, expression of LMP1 was significantly associated with XBP-1 expression in NPC biopsies. It is quite interesting that LMP1 and UPR exert similar oncogenic effects, including activation of NF-kB, prevention from apoptosis, and promotion of angiogenesis. Therefore, our study suggests that ER stress may promote NPC progression through co-induction of UPR and LMP1, providing the first and novel linkage between ER stress and NPC development.

30 (RegID: 1173)
Jillian Brandon
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THE SRC FAMILY KINASE, LYN, NEGATIVELY REGULATES AKT ACTIVATION IN LMP2A-EXPRESSING CELLS

Jillian M. Brandon, Stephanie E. Whittaker, Diana J. Windsor, Sorcha A.M. Collins, and Robert J. Ingham.

Oralabstract:

The EBV protein, LMP2A, is critical for maintaining viral latency and provides pro-survival and migration signals for EBV-positive B and epithelial cell malignancies. LMP2A's ability to initiate signaling was initially proposed to proceed via a two-step mechanism. Firstly, recruitment of the Lyn tyrosine kinase to the tyrosine phosphorylated YEEA site in LMP2A allows for tyrosine phosphorylation of the LMP2A ITAM. This, in turn, facilitates the recruitment and activation of the Syk tyrosine kinase, which initiates downstream signaling events. However, recent findings suggest that Syk could be recruited to LMP2A independent of the YEEA site. Therefore, we undertook experiments to clarify the role of the YEEA motif and Lyn in initiating LMP2A signaling in B lymphocytes. We found that the YEEA site was not required for LMP2A ITAM tyrosine phosphorylation, or for LMP2A to activate Syk. Using siRNA to silence Lyn expression or Lyn-deficient chicken DT40 B cells, it was observed that ITAM and Syk tyrosine phosphorylation did not require Lyn in LMP2A-expressing cells. Furthermore, Lyn was not required for LMP2A-mediated Akt activation in DG75 B cells, but rather Akt activation was significantly enhanced in LMP2A-expressing cells in which Lyn was reduced. We propose that Lyn negatively regulates LMP2A-mediated Akt activation by phosphorylating tyrosine 323 of Syk, which serves to recruit the c-Cbl E3 ubiquitin ligase to Syk and targets Syk for ubiquitin-mediated degradation. In sum, this work demonstrates that LMP2A can initiate signaling independent of Lyn and that Lyn can negatively regulate LMP2A signaling.

NOTES:

Oral speaker
Abstract

Session 6: SIGNAL TRANSDUCTION (Ⅱ)

Chairpersons: Yao Chang, Takeshi Sairenji

Sunday, November 9, 10:40-12:00

31 (RegID:1114; 1115; 1116）

Elena Kashuba
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EBV-ENCODED EBNA-5 BINDS TO MDM2 AND MDMX AND PREVENTS P53-INDUCED GROWTH ARREST AND APOPTOSIS

E. Kashuba1, M. Yurchenko2, SP. Yenamandra1, B. Snopok3, B. Bercovich4, A. Ciechanover4, and G. Klein1

Oralabstract：

We have previously shown that primary EBV infection leads to a striking increase of p53 expression in the transformed immunoblasts that is not associated with apoptosis. Moreover, EBV transformed lymphoblastoid cell lines maintain p53 in the wild type configuration during many years of serial propagation, in contrast to Burkitt lymphoma lines where the p53 pathway was regularly impaired by mutation or functionally corresponding changes. In freshly EBV-infected cells, stainable p53 expression reached maximum after 48 hours, in parallel with EBNA-5 expression. Interestingly, the p53 positive immunoblasts did not express p21. This raised the interesting possibility that the p53 downstream growth arrest and apoptotic pathways may be non-functional.

Recently, we have found that EBNA-5 may protect LCLs from p53-dependent G1 arrest and apoptosis through its binding to MDM2, the E3 ubiquitin ligase that promotes polyubiquitination and subsequent degradation of p53. While studying the interplay of p53, p14ARF, and MDM2 proteins by SPR and GST-pull down assay in EBV-driven LCLs, we have found that EBNA-5 forms a complex with MDM2 and MDMX. To explore the functional consequences of MDM-2 肯 BNA-5 binding, p53 polyubiquitination and degradation assays were performed in vitro. EBNA-5 was found to inhibit MDM2-dependent polyubiquitination (but not monoubiquitination) of p53. The degradation of p53 by 26S proteasomes was also inhibited by EBNA-5. This may explain the high p53 level in LCLs. Noteworthy; p53 is not transcriptionally active in LCL. Treatment of freshly EBV-infected B-cells with DNA damaging agent (mitomycin C) led to increase of p53 protein without parallel upregulation of p21, GADD45, and VDR. In contrast, the above mentioned proteins were upregulated in anti-CD40+IL-4 activated B-cells upon treatment with mitomycin C.

32 (RegID: 1241)
John O'Neil
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EPSTEIN-BARR VIRUS-ENCODED EBNA1 MODULATES THE NF-KB PATHWAY IN CARCINOMA CELLS IN VITRO

R. Valentine, K. M. Shah, J.R. Arrand, C.W. Dawson, L.S. Young and J.D O'Neil

Oralabstract:
The Epstein-Barr virus (EBV)-encoded EBNA1 protein is expressed in all virus-associated tumours, including nasopharyngeal carcinoma (NPC), where it plays an essential role in EBV genome maintenance, replication and transcription. Previous studies suggested that EBNA1 may have additional effects relevant to oncogenesis, including enhancement of cell survival, raising the possibility that EBNA1 may influence cellular gene expression. We have demonstrated in NPC cell models that EBNA1 influences the expression of a range of cellular genes involved in transcription, translation and cell signalling pathways. These studies have revealed a novel role for EBNA1 in modulation of the TGF-beta  and AP-1 signalling pathways and that the latter results in the elevated expression and secretion of angiogenic cytokines and enhanced angiogenesis in vitro.
In the current study we extended our analysis to the NF-B pathway. Here we demonstrate that expression of EBNA1 in a range of carcinoma derived lines resulted in down-regulation of NF-κB reporter activity and reduced expression of several NF-κB regulated genes. Detailed analysis revealed that inhibition of NF-κB was specifically mediated through the inhibition of p65 nuclear translocation in response to the pro-inflammatory cytokine TNF-beta; and IL-1-beta. Failure to translocate was not a consequence of IκBα over-expression; rather, p65 phosphorylation was abrogated which correlated with decreased IKKα/β phosphorylation. As selective inhibition of p65 NF-κB has been documented in NPC our findings further implicate EBNA1 in the pathogenesis of this disease.

33 (RegID: 1249; 1250）
Ellen Cahir-McFarland
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NF-KB SUPPORTS GLYCOLYSIS IN EBV TRANSFORMED LCLS

Thomas Sommermann and Ellen Cahir-McFarland

Oralabstract:
EBV Latent Membrane Protein 1 (LMP1)-mediated NF-kB activation is required for LCL transformation and continued survival. Mutations in LMP1 that diminish the ability to activate NFkB diminish transformation. Further, NFkB inhibitors cause LCLs to die without any other chemotherapy. NF-kB has a clear anti-apoptotic activity through induction of c-IAP1/2, c-FLIP and BCL2 family members. However, NF-kB must support additional, unknown growth and survival pathways because caspase inhibitors do not protect LCLs from NF-kB withdrawal.
Our data indicates NF-kB modulates survival by supporting anaerobic glycolysis in an oxygen rich environment (the Warburg effect). LCLs treated with NF-kB inhibitors reduce lactate and ATP production. Further, NF-kB inhibitors sensitize LCLs to oliogmycin, an inhibitor of the mitochondrial atp synthase, reducing the IC50 from 16uM to 8uM. NF-kB inhibitors change GFP-LC3 location from diffuse to punctate indicative of increased autophagy; and 3-methyl adenine, an autophagy inhibitor, synergizes with NF-kB inhibitors to promote LCL cell death. Thus, NF-kB supports glycolysis, NF-kB inhibitors induce carbon and energy deprivation plus autophagy, and autophagy limits LCL cell death. The mechanisms by which NF-kB enhances glycolysis are under investigation.

34 (RegID: 1739)

Christopher Whitehurst
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THE EBV DEUBIQUITINATING ENZYME, BPLF1, INTERACTS WITH AND REDUCES THE ACTIVITY OF EBV RIBONUCLEOTIDE REDUCTASE.

Christopher B. Whitehurst, Julia Shackelford, Edward Gershburg, Gretchen L. Bentz, and Joseph S. Pagano

Oralabstract:

The first virally encoded deubiquitinating enzyme (DUB) was recently discovered in HSV and is strictly conserved across Herpesviridae. No protein targets of the herpesviral DUBs have been identified. EBV DUB activity is exhibited by BPLF1, a large tegument protein (3149 AA) expressed in the lytic cycle. Yeast-2-hybrid screening had predicted an interaction between BPLF1 and the EBV ribonucleotide reductase small subunit (BaRF1 or RR2). To investigate the interaction of BPLF1 and RR2, a functional clone composed of the first 246 N-terminal amino acids of BPLF1 (BPLF1 1-246) that expresses EBV DUB activity was constructed. Co-immunoprecipitation verified an interaction between RR2 and EBV DUB. We first found that the large (RR1) but not the small (RR2) subunit of the ribonucleotide reductase was ubiquitinated. Ubiquitination of RR1 only occurred when both subunits were expressed together. When RR1 and RR2 were co-expressed in the presence of EBV DUB, the ubiquitinated form of RR1 was no longer detected. Studies of the cellular topology of the proteins verify that EBV RR1, RR2, and the N-terminal fragment of BPLF1 co-localize to the cytoplasm in 293T cells. Studies to determine if EBV DUB activity affects viral EBV RR revealed that a decrease in EBV reductase activity resulted when EBV DUB was present. Significant loss in RR activity was not observed with a non-functional DUB mutant (BPLF1 C61S) suggesting that the DUB activity of BPLF1 is responsible for down-regulation of EBV RR activity. These results indicate that the N-terminal portion of BPLF1 interacts with, deubiquitinates, and regulates the activity of viral RR. This is the first verified target of EBV DUB activity.

NOTES:

Oral speaker
Abstract

Session 7: NASOPHARYNGEAL CARCINOMA

Chairpersons: Kai-tai Yao, Qian Tao

Sunday, November 9, 14:20-16:00

35 (RegID:1170; 1171; 1172)

Joanna Wilson
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MASSIVE OVER-EXPRESSION OF CHITINASE3-LIKE4 (YM2), CD30 AND IMMUNOGLOBULIN IN LMP1 INDUCED INFLAMMATION-ASSOCIATED CARCINOGENESIS

Adele Hannigan, P. Monica Tsimbouri and Joanna B. Wilson

Oralabstract:

There is an increasing body of evidence linking chronic inflammation and cancer but the mechanistic connection is poorly understood and the mediators of inflammation immensely complex. Most cancers are accompanied by leukocyte infiltration (and nasopharyngeal carcinoma is no exception), which, contrary to the anticipated immune role, could be contributing to tumour development and progression. In order to explore key carcinogenic factors in EBV associated malignancy we have studied a transgenic mouse model of epithelial carcinogenesis in which the Epstein-Barr virus oncogene LMP1 (CAO variant) is expressed. The preneoplastic skin, demonstrating hyperplasia and erosive dermatitis, was found to be inflamed with a mixed infiltrate involving T-cells (including NKT cells), mast cells and neutrophils. Several inflammatory factors were upregulated in the affected tissue, notably CD30 and CD30L, also MIP2, MIP3, CRG2/IP-10 and IL-1β and particularly the B-cell attractant CXCL13 (BLC). Immunoglobulin deposition was found to occur as the pathology worsens indicating a B-cell involvement. The role of mature T- and/or B-lymphocytes in the advancing pathology was demonstrated by their elimination, which limits the pathology to an early, benign stage. We have found that a chitinase-related protein, lacking enzymatic activity (termed YM2 or chitinase3-like4) is massively upregulated in the LMP1 expressing epidermis. We will present evidence to suggest that YM2 may be acting in concert with immunoglobulin to promote inflammation. The repeated identification of over-expressed chitinase-like proteins in models of inflammatory disorders suggests a crucial role for this protein family in inflammation. Factors which promote or sustain chronic inflammation may prove to be effective chemotherapeutic targets and their identification is therefore an important prerequisite. In this model LMP1 is the inducing factor and its effect upon and purturbation of immune system cells (of both innate and adaptive systems) leading to an inflamed state may augment its oncogenic activity in the development of EBV associated carcinoma.

36 (RegID: 1015)
Chin-Tarng Lin
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NASOPHARYNGEAL CARCINOMA (NPC): MOLECULAR PATHOGENESIS AND CLINICAL INTERVENTION

Chin-Tarng Lin
Postgraduate Institute and Department of Pathology, College of Medicine, National Taiwan University and Hospital, Taipei, Taiwan

Oralabstract:
NPC is one of the common cancers among Chinese living in South China, Taiwan, Singapore and other countries. The etiological factors have not been clearly identified yet. So far, no any major gene related with hereditary factor has been identified in NPC carcinogenesis; however, some environmental factors, such as consumption of salted fish and Chinese herbs in Hong Kong and long term exposure to the sulfuric acid vapor in Taiwan have been suspected to be related to NPC induction, while Epstein-Barr virus (EBV) has been proposed to be closely associated with NPC pathogenesis. To investigate the relationship between NPC and EBV, we have established and characterized ten NPC cell lines. After extensive investigation about the relationship between NPC and EBV, we conclude that EBV can establish an infection only in nasopharyngeal neoplastic cells but not nasopharyngeal metaplastic epithelial cells, which led us to use the IgA receptor mediated endocytosis of EBV-IgA-SC method to infect NPC cells. These observations imply that EBV may play more important role in the tumor progression stage rather than in the tumor initiation stage of the NPC carcinogenesis process. Furthermore, using the in vitro and in vivo systems to analyze the response of NPC host genes to EBV infection, we found that EBV can not turn on any host gene which is not expressed in NPC cells, but can enhance the gene expression in tumor cells which express this gene. These investigations consolidate and expand our hypothesis that EBV may play an important role in enhancement of NPC progression but not involve in the initiation and promotion stage of NPC pathogenesis. This is of fundamental importance to our concept of the EBV-associated human neoplasm and may provide basis for the prevention and therapy of NPC.

37 (RegID: 1062)

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FUNCTIONAL CHARACTERIZATION OF A CHROMOSOME 11 CANDIDATE TUMOR SUPPRESSOR GENE, THY1, IN NASOPHARYNGEAL CARCINOMA

HL Lung, AKL Cheung, Y Cheng, FM Kwong, PHY Lo, EWL Law, D Chua, ER Zabarovsky, N Wang, SW Tsao, EJ Stanbridge, ML Lung

Oralabstract:

Previous studies on identifying chromosome 11 critical regions associated with functional suppression of tumor formation in nasopharyngeal carcinoma (NPC) utilized a monochromosome transfer and complementation approach, which successfully identified several candidate tumor suppressor genes (TSGs) mapping to chromosome 11q22-q23. These include CADM1 (Cell Adhesion Molecule 1, formerly known as TSLC1), THY1 (THY1 cell surface antigen), and CRYAB (crystalline alpha B). Our previous studies suggested that THY1 is a good candidate TSG associated with lymph node metastases in NPC. To examine the tumor-suppressive activity of THY1, we have now used the in vivo tumorigenicity assay, performed using a tetracycline-regulated expression vector system. Tumorigenicity results show that the activation of THY1 suppresses tumor formation of HONE1 cells in nude mice. Tumor formation ability was restored in the presence of doxycycline, when the gene is selectively shut off. Additional functional studies were performed and results indicate expression of THY1 inhibits HONE1 cell growth in vitro, induces cell cycle arrest, reduces anchorage-independent growth, and inhibits cell invasiveness. An interesting novel link between THY1 and our other two chromosome 11q23 candidate tumor suppressors, CADM1 and CRYAB, indicate there may be potential collaborative interactions of these closely mapping genes. Gene expression levels of CADM1 and CRYAB appear to be tightly regulated by THY1 in HONE1 cells. This possibility is currently being tested.

38 (RegID:1234)
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MOLECULAR GENETIC ANALYSIS OF NASOPHARYNGEAL CARCINOMA

Chunfang Hu, Xiaoyi Chen, Wenbin Wei, Andrew I Bell, John Nicholls, Yunhong Yao, Irène Joab, Paul G Murray, Lawrence S Young, Chris W Dawson and John R Arrand.

Oralabstract:

MOLECULAR GENETIC ANALYSIS OF NASOPHARYNGEAL CARCINOMA

Chunfang Hu1, Xiaoyi Chen1,2, Wenbin Wei1, Andrew I Bell1, John Nicholls3, Yunhong Yao1,2, Irène Joab4, Paul G Murray1, Lawrence S Young1, Chris W Dawson1 and John R Arrand1.

1Cancer Research UK Institute for Cancer Studies, School of Cancer Sciences, University of Birmingham, Birmingham, B15 2TT, U.K.; 2Department of Pathology, Guangdong Medical College, Zhanjiang, Guangdong, China; 3Department of Pathology, The University of Hong Kong, Hong Kong, China; 4UMR542 Inserm-Université Paris Sud, Hôpital Paul Brousse, Villejuif, France.

Carcinogenesis commonly involves extensive genetic changes and perturbation of cellular gene expression profiles in tumour cells. Here we describe an analysis of these phenomena in the EBV-associated neoplasm, nasopharyngeal carcinoma (NPC). Snap-frozen biopsies of EBV-positive NPC were obtained from Southern China and the Maghreb region of North Africa. Tumour cells and cells from normal epithelium were obtained by laser-capture microdissection of frozen sections. DNA and total RNA were extracted, amplified and analysed using Affymetrix 500K SNP arrays and U133Plus2 expression arrays. Examination of tumour cell DNA revealed that in addition to several extensive regions of chromosomal gain and loss the high resolution of the SNP arrays identified a number of stretches of relatively short, discrete copy-number changes. Quantitative PCR was used to validate the array predictions. Loss of heterozygosity analysis revealed that several tumours exhibited uniparental disomy on some chromosomes in addition to a number of smaller homozygous regions. Gene expression analysis of tumour cells compared with normal nasopharyngeal epithelial cells showed that the levels of expression of a large number of transcripts were significantly altered. Neither the SNP data nor the expression profiles suggested any substantial geographically-related differences between samples. The expression data were analysed in the context of genomic copy number changes in the same tumour and for alterations in known oncogenes and tumour suppressor genes. The data are consistent with our earlier observations of downregulation of HLA Class I expression in NPC and the induction of RNA polymerase III-related transcription factors by EBV. In addition we have identified alterations in key signalling pathways that regulate epithelial cell growth and differentiation. In agreement with earlier studies we observe alterations in the canonical and non-canonical Wnt signalling pathways and in addition have identified alterations in the TGF-beta pathway.

39

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BMI-1, A POLYCOMB GROUP PROTEIN, PLAYS AN ESSENTIAL ROLE IN TUMORIGENESIS AND METASTASIS

Li-Bing Song1,2, Jun Li1,2, Wen-Ting Liao1.2,Yan Feng1.2, Man-Zhi Li1.2, Yi-Xin Zeng1.2 and Mu-Sheng Zeng1.2# 1State Key Laboratory of Oncology in Southern China and 2Departments of Experimental Research, Sun Yat-sen University Cancer Center, Guangzhou, China. 510060.

Oralabstract:

Metastasis is the major cause of mortality in most cancer patients, but the underlying molecular mechanisms are largely unknown. The Polycomb group (PcG) proteins are epigenetic gene silencer proteins that play key roles in embryonic development and oncogenesis. Our recent studies and many other's indicate that dysregulation of PcG proteins correlate with invasive and metastatic phenotype of various human cancer types for which the underlying mechanism is not clear. Here, we report that ectopic expression of the polycomb protein Bmi-1 causes epithelial to mesenchymal transition (EMT), enhances cell motility/invasiveness, and induces tumorigenesis and metastasis, whereas silencing Bmi-1 expression in cancer cells reverses EMT and reduces metastatic potential. We further demonstrate that the AKT/GSK-3β/Snail cascade is dysregulated in Bmi-1 overexpressing cells. Most importantly, there is an inverse correlation between Bmi-1 expression and E-cadherin expression in human cancer specimens. This study provides novel functional and molecular mechanistic links between Bmi-1 and cancer progression, rendering Bmi-1 a potential target for therapeutic intervention. In addition, this study opens a new outlook for understanding the functional correlation of PcG proteins in tumor progression, and provides insight into the process of tumor metastasis in cancer patients.

Keywords: Bmi-1; Polycomb group protein; epithelial to mesenchymal transition; Metastases

NOTES:

Oral speaker
Abstract

Session 8: **BURKITT'S LYMPHOMA**

Chairpersons: **Evelyne Manet, Denis Moss**

Sunday, November 9, 16:55-18:15

40 (RegID: 1306; 777）
Gemma Kelly
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AN EPSTEIN-BARR VIRUS ANTI-APOPTOTIC PROTEIN CONSTITUTIVELY EXPRESSED IN TRANSFORMED CELLS AND INVOLVED IN BURKITT LYMPHOMAGENESIS: THE BAMHI W PROMOTER/BHRF1 CONNECTION

Gemma L. Kelly, Heather M. Long, Julianna Stylianou, Wendy A. Thomas, Andrew I. Bell, Georg Bornkamm, Josef Mautner, Alan B. Rickinson and Martin Rowe

Oralabstract:

Two factors contribute to the pathogenesis of Burkitt lymphoma (BL), infection with EBV and a chromosomal translocation leading to c-myc oncogene deregulation. The role of EBV in BL remains unclear since the virus' growth transforming program is not expressed. Instead EBV in BL normally exhibits a restricted Latency I form of infection characterised by expression of only one latent antigen EBNA1 from the Qp promoter. Recently we identified a subset of 15% of BLs where tumourigenesis has selected for a rare EBNA2-deleted virus mutant. These tumours use Wp rather than Qp and, in addition to EBNA1, express EBNAs 3A, 3B, 3C and a truncated –LP. This Wp-restricted latency provides unusually strong resistance to apoptosis, strengthening the view that EBV serves to counteract the high apoptotic sensitivity which is a consequence of deregulated c-myc expression.

Using an inducible system to express the candidate antigens in a BL cell, we show that this apoptosis resistance is mediated not by one of the extended range of EBNAs seen in Wp-restricted latency but by Wp-driven expression of the viral bcl2 homologue, BHRF1, a protein usually associated with lytic cycle. Interestingly this Wp/BHRF1 connection is not confined to Wp-restricted BLs but appears integral to normal B cell transformation by EBV. Thus we find that the BHRF1 gene expression recently reported in newly-infected B cells is temporally linked to Wp activation. Furthermore, just as Wp remains detectable in LCLs, these BHRF1 transcripts are expressed long-term. Most importantly, using the sensitivity of BHRF1 epitope-specific T cells, we confirm that the protein is constitutively expressed in LCLs made using a replication deficient BZLF1-KO virus. Thus this work provides evidence that the EBV bcl2 homologue is involved in virus-associated lymphomagenesis in vivo and establishes BHRF1 as a tenth latent cycle protein constitutively expressed in transformed cells in vitro.

41 (RegID:1133; 1134）

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EFFECTS OF HOLOENDEMIC MALARIA ON PRIMARY EBV INFECTION IN CHILDREN FROM WESTERN KENYA

Erwan Piriou1, Amolo Asito2, Nancy Fiore1, Peter S. Odada2, Jaap M. Middeldorp3, Ann M. Moormann4, Robert Ploutz-Snyder1, Rosemary Rochford1.

Oralabstract:

Early age of EBV infection as well as repeated Plasmodium falciparum infections are etiologically linked to endemic Burkitt's lymphoma (eBL). However, it is unknown whether and how malaria affects primary EBV infection in infants. To study the kinetics of acquisition of EBV in relation to malaria, two groups of children are being followed longitudinally from birth through 3 years of age. The first group is from an area with holoendemic P. falciparum (Kisumu District, Kenya) and a high incidence of eBL, whereas the second group is from an area with sporadic P. falciparum transmission (Nandi District, Kenya) and a low risk for eBL. EBV DNA levels were determined in whole blood isolated from infants at 1 month through 18 months of age in 96 children from Kisumu and 106 children from Nandi. More children were infected earlier in life in Kisumu with a median age of infection of 10 months and 90.4% infected by 18 months, in comparison to 12 months and 65.4% infected by 18 months in Nandi. Following primary infection, peak levels in EBV DNA were comparable in both sites. However EBV viral load continued to increase over time in children from Kisumu, whereas they decreased and remained low after the first peak in children from Nandi suggesting that the malaria is not altering the establishment of EBV latency but the maintenance of latency. EBV serology was done on a subset of the study population. We found that the appearance of EBV DNA preceded or matched the appearance of EBV-specific IgG indicating that viral load is a sensitive marker of primary infection. Together, this data suggests that the pattern of EBV infection is already altered early in life in children from an area with holoendemic malaria possibly setting the stage for the emergence of malignancy.

*This research was funded by NIH R01 CA102667 to RR

42 (RegID: 1768; 1769)
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THE EPSTEIN-BARR VIRUS BART MIRNAS PROVIDE A SURVIVAL ADVANTAGE TO BURKITT'S LYMPHOMAS

David Vereide and Bill Sugden

Oralabstract:

Multiple Burkitt's lymphoma (BL) cell lines harbor EBV for many generations following explantation, indicating that EBV provides a selective advantage to these cells proliferating in culture. We have sought to understand this selective advantage by forcing the loss of EBV from cells using an inducible dominant negative derivative of EBNA1. We find in two Type I BL cell lines, SavI and Dante, that the forced loss of EBV correlates with the cells failing to survive. Populations of cells losing the virus have a lengthened doubling time and are prone to apoptosis. We have found that the ectopic expression of the BART miRNAs rescues the inhibition of survival. Using microarrays, we have measured levels of cellular mRNAs in BART-complemented and uncomplemented cells that have lost EBV to examine the mechanism by which BART miRNAs exert their pro-survival function. These analyses have been coupled to ones measuring the induction of expression of cellular genes following viral infection of primary B-cells. These measurements have identified cellular genes whose expression is inhibited upon infection of primary B-cells and stimulated upon the loss of EBV. We are now testing these cellular candidates to determine whether they are regulated directly or indirectly by the BART miRNAs to affect cell survival.

43 (RegID: 1760; 1761; 1763)

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CO-TRANSPLANTED HUMAN THYMUS IMPROVES CONTROL OF EPSTEIN-BARR VIRUS INFECTION IN A HUMANIZED-SCID-GAMMA C-/- MOUSE MODEL, AND LYTIC VIRAL INFECTION INCREASES LYMPHOMA NUMBER.

Shidong Ma, Deepika Rajesh, Ruth Sullivan, William J. Burlingham, Xiaoping Sun, Margaret L. Gulley, and Shannon C. Kenney

Oralabstract:

NOD/SCID/gamma C-/- mice injected with human CD34+ hematopoitic stem cells provide a model for studying EBV pathogenesis in the context of a human immune system. Whether transplanted human thymic tissue improves control of EBV infection, and whether lytic EBV infection increases lymphomas, in this model remains unclear. We compared the effects wild-type EBV infection, versus infection with a lytic-defective mutant (BZLF1-deleted) in mice injected with human fetal CD34+ cells in the presence or absence human thymus tissue. Mice were injected intraperitoneally with 2700 infectious units of wt or lytic-defective B95-8 virus. In the absence of human thymus tissue, all EBV infected mice developed polyclonal B cell lymphomas (some with type III viral latency and some with EBNA2+, LMP1-neg. tumors), and wildtype and lytic-defective EBV had similar effects. In the presence of co-transplanted human thymic tissue, approximately half of the animals (6/11) infected with wt virus developed lymphomas. In contrast only 13% (2/15) of animals infected with lytic-defective virus had tumors. Tumors were a mixture of type III, type II and type I (both polyclonal and monoclonal). In animals without lymphoma, EBER+ B cells with type I latency were found in similar numbers, and similar locations (including lymph nodes, spleen, kidney, omentum, lung, peri-nasal lymphoid tissue, and bone marrow), in wt and mutant virus infected animals, and rare cells expressed EBNA2. In wt virus-infected animals, rare BZLF1-expressing cells were found in B cells, as well CD68-positive monocytes. These results suggest that 1) the presence of co-transplanted human thymic tissue helps human T cells to control EBV infection in this new mouse model, and 2) lytic EBV infection is not absolutely required for development of B cell lymphomas in this model, at least when a relatively high titer of virus is administered, but may contribute in the presence of the thymus.

NOTES:

Oral speaker
Abstract

Session 9: OTHER MALIGNANCIES AND PTLD

Chairpersons: Shannon Kenney, Lori D Frappier

Monday, November 10, 08:55-09:55

44 (RegID: 1453)
Hideyuki Ishii
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THE INVESTIGATION OF INTERACTION BETWEEN NASAL NK/T-CELL LYMPHOMA AND THE INFLAMMATION CELL.

Hideyuki Ishii, Miki Takahara, Yasuaki Harabuchi, Eva Klein

Oralabstract:
Background: Nasal NK/T-cell lymphoma (NNKTL) is the lymphoproliferative disorder with necrotic granulomatous legions in the central part of head and neck region such as nasal cavity and hard palate. It is known Epstein–Barr virus (EBV) associates with etiology of this lymphoma and is detectable in the tumor cells.This lymphoma is characterized by the remarkable necrosis formation and the extensive infiltration of inflammation cells in tumor tissue histopathologically. In terms of these, we investigate the interactions between inflammation cells and NNKTL in this study.
Methods: Monocyte and granulocyte were separated from buffy coat of the volunteers as inflammation cells. Each of these cells was co-cultured with SNK6 (NNKTL cell line) and KAI3 (EBV positive NK cell line) without IL-2. Proliferation and LMP1 expression were compared with these lines (SNK6, KAI3) without monocyte culture with/without IL-2. Finally, we investigated CD14 expression of 20 NNKTL patients tissues by immunohistochemistry.
Results: CD14 expression was detected all NNKTL patient tissue. SNK6 and KAI3 showed the proliferation under the condition that they co-cultured with monocyte without IL-2. LMP1 expression of these cells increased at the same time. SNK6 and KAI3 did not show any proliferation under the condition that they co-cultured with granulocyte without IL-2. LMP1 expression of these cells did not increase. SNK6 and KAI3 did not show any proliferation under the condition that they co-cultured with monocyte without cell-to-cell contact without IL-2. LMP1 expression of SNK6 and KAI3 did not increase at the same time.
Conclusions: This study suggested that monocyte is one of the factors that influence to NNKTL proliferation. It is possible for NNKTL to proliferate without IL-2, and cell-to-cell contact of monocyte and NNKTL cell was needed to give rise to this phenomenon.

45 (RegID: 1495)
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MECHANISMS UNDERLYING THE SELECTIVE IMPAIRMENT OF EBNA1-SPECIFIC EFFECTOR T-CELLS OBSERVED IN PTLD.

K. Jones, J. Nourse,S. Yekollu, L. Morrison, D. Moss, MK Gandhi.

Oralabstract:

Immunosurveillance by cytotoxic T lymphocytes (CTL) plays a critical role in the detection and killing of lymphoma. The ability to evade CTL recognition promotes cancer survival. In EBV-driven PTLD, evasion was believed to be simply the result of iatrogenic immunosuppression leading to an absence of EBV-specific cellular surveillance. If this were true, EBV-specific immunity would be "globally" depressed. Conversely if lymphoma-driven mechanisms operate this would result in selective impairment against only those EBV-antigens expressed within the diseased lymph node. Although EBV-latent antigen expression in PTLD is variable, EBV-nuclear antigen 1 (EBNA1) is universally expressed. EBNA1 may offer a viable antigenic target for the treatment of PTLD. A deeper understanding of the mechanisms utilized by the lymphoma cell to evade EBNA1 is needed to optimize immunotherapeutic strategies.

CTL capable of proliferation and interferon-gamma (IFN-gamma) and/or CD107 granule release were studied in 12 PTLD patients. Blood was taken prior to therapy and results compared with 19 healthy EBV-seropositive controls. T-cells were expanded using peptide-pools directed against the EBV-antigens EBNA1 or the lytic protein BZLF1 (the latter is not expressed in PTLD) in a 14-day culture and assayed by flow cytometry.

Strikingly, we observed a four-to-five fold reduction in both IFN-gamma and CD107 releasing EBNA1-specific CTL in PTLD patients as compared to controls. By contrast, BZLF1-specific CTL were unimpaired relative to controls, consistent with a lymphoma-specific inhibition mediated within the tumour microenvironment. Class I pentamer EBNA1-peptide-specific T-cell lines showed strong expression of the T-cell inhibitory receptor PD1 and concomitant expression of its ligands PDL1/2 on EBV-transformed cell-lines and 16 spontaneously outgrown primary patient samples.

We demonstrate a selective impairment of immunity against a highly-relevant tumour-associated antigen and suggest that EBNA1-specific CTL are under the inhibitory influence of PDL1/2 expressed by malignant B-cells. Our finding provides a new potential strategy for the treatment and prevention of PTLD.

46 (RegID: 1270)

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CLINICOPATHOLOGIC FEATURES AND EBV GENOME POLYMORPHISM OF EPSTEIN-BARR VIRUS-ASSOCIATED GASTRIC CARCINOMA IN GUANGZHOU

Chun-Kui Shao, Jian-Ning Chen, Yun-Gang Ding

Oralabstract:

Objective: To investigate the clinicopathologic features and EBV genome polymorphism of Epstein-Barr virus-associated gastric carcinoma (EBVaGC) in Guangzhou, an endemic area of nasopharyngeal carcinoma (NPC).

Materials and methods: In situ hybridization assay of EBV-encoded small RNA-1 (EBER-1) was used to identify the presence of EBV in 676 consecutive gastric carcinoma cases from the 2nd and 3rd Affiliated Hospital of Sun Yat-sen University, and Guangzhou First Municipal People's Hospital, from 2000 to 2006. EBV encoded proteins EBNA1, EBNA2, LMP1 and ZEBRA were detected by immunohistochemistry. EBV genome polymorphism was also analyzed.

Results: Forty-five EBV-positive cases (6.7%) were identified, including 37 (8.5%) male and 8 (3.3%) female cases. The proportion of EBVaGCs decreased with age (P = 0.003). EBVaGCs accounted for 11.5% (15/130) of carcinomas in the cardia, 10.2% (14/137) in the middle stomach, 3.5% (14/400) in the antrum, and 22.2% (2/9) in the whole stomach. The proportion of EBVaGCs in diffuse-type carcinomas was higher than that in intestinal-type ones (8.3% vs. 3.5%; P=0.039). EBNA1 was detected in 42 cases (93.3%), while EBNA2, LMP1 and ZEBRA were all negative. For EBV genome polymorphism analysis, all cases were type 'I'. Wild-type 'F' and variant-type 'f' accounted 37 and 3 of the cases, respectively. There were also 5 cases with both wild-type 'F' and variant-type 'f'.

Conclusion: The present study not only described the frequency and clinicopathologic features, but also for the first time revealed the EBV latency pattern and EBV genome polymorphism of EBVaGC in Guangzhou. The EBV latency pattern in EBVaGC is type Ⅰ. The predominance of type 'I' strain in EBVaGC is similar to that in NPC, while predominant type 'F' in EBVaGC is apparently different from that in NPC in Guangzhou, suggesting that distinct EBV strains may be related to the development of different EBV associated carcinomas.

NOTES:

Oral speaker
Abstract

Session 10: IMMUNE MECHANISMS, VACCINE AND IMMUNOTHERAPY

Chairpersons: Maria Masucci, Jaap Middeldorp

Monday, November 10, 10:10-12:30

47 (RegID: 1716)
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ACTIVATION AND EXPANSION OF PROTEIN-SPECIFIC T-CELLS BY UREA FORMULATED PROTEINS OR PEPTIDES: IMPLICATION FOR DIAGNOSTIC, PREVENTIVE AND THERAPEUTIC APPLICATIONS

Deml, L.; Barabas, S.; Edmaier, K.; Wolf, H.

Oralabstract:

Soluble extracellular proteins usually do not enter the endogenous human leukocyte antigen (HLA) I-dependent presentation pathway of antigen-presenting cells, strictly impeding their applicability for the re-stimulation of protein-specific CD8+ cytotoxic T-lymphocytes (CTL). Here we present for the Epstein Barr-Virus (EBV) BZLF1 a novel strategy that facilitates protein translocation into antigen-presenting cells by its solubilisation in high molar urea and subsequent pulsing of cells in presence of low molar urea. Stimulation of PBMC from HLA-matches EBV-seropositive individuals with urea-treated BZLF1 but not untreated BZLF1 induces an efficient reactivation of BZLF1-specific CTL. Urea-treated BZLF1 (uBZLF1) enters antigen-presenting cells in a temperature-sensitive manner through clathrin-mediated endocytosis and is processed by the proteasomes into peptides that are bound to nascent HLA I molecules. Dendritic cells and monocytes but also B-cells can cross-present uBZLF1 in vitro. The strategy described here has potential for use in the development of improved technologies for the monitoring of protein-specific CTL and may facilitate a novel strategy for the expansion of protein-specific CTL for novel diagnostic read-outs as well as for therapeutic or preventive applications.

48 (RegID: 1149; 1150; 1151)

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EPSTEIN-BARR VIRUS-ENCODED BILF1 CONTRIBUTES TO IMMUNE EVASION BY TARGETING MHC CLASS I FOR DEGRADATION

Jianmin ZUO, Andrew CURRIN, Wendy A THOMAS and Martin ROWE

Oralabstract:

Recent evidence indicates that an important component of the ability of EBV to establish an asymptomatic persistent infection in healthy individuals involves active interference with the MHC class I antigen processing pathway during the lytic EBV replication cycle. Previously, BNLF2a and BGLF5 proteins were shown to interfere with antigen presentation by respectively impairing TAP-mediated peptide transport and by initiating host protein synthesis shut-off to reduce expression of MHC class I proteins. We have now identified a novel role for the lytic cycle gene, BILF1, which encodes a glycoprotein with the properties of a constitutive signaling G-protein-coupled receptor (GPCR). BILF1 reduced the levels of MHC class I at the cell surface and inhibited CD8+ T cell recognition of endogenous target antigens. The underlying mechanism involves physical association of BILF1 with MHC class I molecules, an increased turnover from the cell surface, and ubiquitination-independent degradation via lysosomal proteases. The immune-modulating and the GPCR-signaling properties BILF1 are distinct and genetically separable functions. Furthermore, surface MHC class I was similarly downregulated by the BILF1 protein of the closely related CeHV15 gamma-herpesvirus of the Rhesus Old World primate (80% amino acid sequence identity). In contrast, levels of surface MHC class I were unaffected by the most closely related human herpesvirus vGPCR, encoded by the ORF74 of KSHV (15% amino acid sequence identity with EBV BILF1). The discovery of a third EBV lytic cycle gene that cooperates to interfere with MHC class I antigen processing underscores the importance of the need for EBV to be able to evade CD8+ T cell responses during the lytic replication cycle, at a time when such a large number of potential viral targets are expressed.

49 (RegID: 1883; 1884; 1885)

Pierre BUSSON
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BLOOD DIFFUSION AND TH1-SUPPRESSIVE EFFECTS OF GALECTIN-9 CONTAINING EXOSOMES RELEASED BY EPSTEIN-BARR VIRUS-INFECTED NASOPHARYNGEAL CARCINOMA CELLS

Jihène Klibi 1, Toshiro Niki 2, Alexander Riedel3, Catherine Pioche-Durieu1, Mitsuomi Hirashima4, Fethi Guemira 5, Sylvestre Le Moulec 6, Joël Guigay 1, Dinesh Adhikary3, Josef Mautner 3, Pierre Busson 1

Oralabstract:

Although malignant cells from EBV-associated nasopharyngeal carcinoma consistently express several immunogenic viral proteins, their strategy of immune escape has remained poorly understood so far. We have previously reported that NPC cells express high levels of galectin 9 which is a ligand of the Tim3 receptor, a death-inducing receptor expressed by mature Th1 lymphocytes. In vitro studies have shown that malignant NPC cells release exosomes containing galectin 9 in the extra-cellular medium (Keryer-Bibens et al., BMC Cancer 2006). Subsequently, we sought to determine whether galectin-9 carrying exosomes were produced in NPC patients and whether such exosomes might play a role in the immune evasion of NPC cells. We report that galectin-9 containing exosomes are selectively detected in plasma samples from NPC patients and mice xenografted with NPC tumors. The incorporation into exosomes protects galectin-9 against proteolytic cleavage but retains its Tim-3-binding capacity. Importantly, NPC exosomes induce massive apoptosis in EBV-specific CD4+ cells used as a model of target T-cells. This effect is inhibited by both anti-Tim-3 and anti-galectin-9 blocking antibodies. These results indicate that blocking galectin-9/Tim-3 interaction in vivo might alleviate the Th1 suppressive effect of NPC exosomes and sustain anti-tumoral T cell responses, and thereby improve clinical efficacy of immunotherapeutic approaches against NPC.

50 (RegID: 1252)

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THE EFFECTS OF ACUTE MALARIA ON EBV LOAD AND EBV-SPECIFIC T CELL IMMUNITY IN GAMBIAN CHILDREN

R. Njie, A. Bell, H. Jia, D. Croom-Carter, S. Chaganti, A. Hislop, H. Whittle and A. Rickinson

Oralabstract:

Endemic Burkitt lymphoma (BL) is the commonest cancer of childhood in equatorial areas of Africa and is consistently EBV genome–positive. In addition to EBV, epidemiologic evidence also strongly implicates high exposure to Plasmodium Falciparum malaria as a second risk factor for developing endemic BL. However it is unclear whether malarial infection might pre-dispose the host to endemic BL through acting as a chronic stimulus to the B cell system and/or through a suppression of EBV-specific T cell surveillance. In this study, we have analysed the effect of acute malaria on the EBV-host balance using Q-PCR assays to determine virus genome load in peripheral blood mononuclear cells and interferon-gamma Elispot assays to enumerate virus-specific CD8+ T cells. We found that Gambian children, sampled during an acute malaria attack and again up to 6 weeks later, sustained extremely high virus loads, reaching levels similar to those seen in infectious mononucleosis patients in the UK. Gambian controls (children and adults with no recent history of malaria) had lower viral loads, though these were still >10-fold above the median for healthy UK adults. Limited studies with EBV epitope peptides (restricted through the HLA-B*3501 and -B*5301 alleles) also suggested an impairment of virus-specific CD8+ T cell function in malarial children, but only during acute disease. The results indicate that acute malaria is associated with a sustained increase in EBV load and, possibly, a transient decrease in EBV-specific T cell surveillance. However, whether these two phenomena, increased EBV load and T cell impairment, are causally linked remains to be resolved. We infer that the unusually high EBV loads in P. falciparum-challenged populations, allied to the parasite’s capacity to act as a chronic B cell stimulus, are likely contributory factors in endemic BL pathogenesis.

51 (RegID: 1725)

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BARF1 AS NEW IMMUNOTHERAPEUTIC TARGET FOR NASOPHARYNGEAL CARCINOMA

D. Martorelli, K. Houali, L. Caggiari, E. Vaccher, L. Barzan, G. Franchin, A. Gloghini, A. Pavan, A. Da Ponte, R.M. Tedeschi, V. De Re, A. Carbone, T. Ooka, P. De Paoli, R. Dolcetti.

Oralabstract:

Immunotherapy approaches targeting Epstein-Barr virus (EBV)-encoded antigens induce objective clinical responses only in a fraction of patients with undifferentiated nasopharyngeal carcinoma (UNPC). In the present study, we have characterized the immunogenicity of the EBV-encoded BARF1 oncogene with the aim to assess whether this protein could constitute a new target antigen for immunotherapy in this setting. Spontaneous CD4+ and CD8+ T cell responses specific for the recombinant p29 BARF1 protein were detected by IFNg-ELISPOT in both EBV-seropositive donors and UNPC patients, but not in EBV-seronegative individuals. Using immunoinformatic prediction tools, we have selected five different candidate BARF1 T cell epitopes presented by HLA-A*0201. Although only one of these peptides was able to bind HLA-A2 with low affinity in the T2 stabilization assay, all five BARF1 nonamers readily elicited specific CD8+ T cell responses in EBV-seropositive HLA-A*0201+ donors and UNPC patients. Notably, the magnitude of CD8+ T cell responses to the whole BARF1 protein and derived A*0201 peptides was significantly higher in UNPC patients than in healthy donors. Moreover, cytotoxic T lymphocytes specific for the p2-10, p23-31, or p49-57 BARF1 peptides were easily obtained from HLA-A*0201+ donors. These cultures were not only able to lyse autologous targets loaded with the antigenic peptide, but also recognized tumor cells endogenously expressing BARF1 in an antigen-specific and HLA-A2-restricted manner. These findings, indicate that BARF1 is a particularly attractive antigen with immunogenic properties in most UNPC patients and provide valuable information to develop new strategies to improve the efficacy of EBV-targeting immunotherapy of UNPC patients.

52 (RegID: 1201; 1202)

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STAGE-SPECIFIC INHIBITION OF MHC CLASS I PRESENTATION BY THE EPSTEIN-BARR VIRUS BNLF2A PROTEIN DURING VIRUS LYTIC CYCLE

Nathan CROFT, Claire SHANNON-LOWE, Andrew BELL, Danielle HORST, Maaike RESSING, Emmanuel WIERTZ, Martin ROWE, Alan RICKINSON, Andrew HISLOP

Oralabstract:

Herpesviruses encode many potential immune targets but have evolved strategies to evade immune recognition. We recently identified an EBV lytic cycle protein, BNLF2a, which abrogates CD8+ T cell recognition of target cells when co-expressed with indicator antigens in these cells. The present experiments sought to determine the influence of BNLF2a in the natural context of lytic EBV replication. Initially we showed that, during lytic cycle, BNLF2a is expressed in the first wave of early gene products at a time when there is a dramatic increase in immediate early and early protein synthesis. We next generated a recombinant BNLF2a knockout EBV and used this to create transformed B-lymphoblastoid lines (LCLs). Cells spontaneously entering lytic cycle in these lines were used as targets in CD8+ T cell recognition assays, where these LCLs were incubated with effectors specific for immediate early, early or late lytic cycle antigens. In the absence of BNLF2a, a striking increase in the recognition of immediate early and at least some early lytic cycle proteins was found when compared with responses made to wild-type EBV transformed LCLs. By contrast, when using late-specific effectors in these assays, no increase in recognition of BNLF2a-deleted targets was observed compared to wild-type targets. Consistent with this functional data, LCLs made with the BNLF2a-deleted virus showed restored surface MHC class I levels during initial replication phases, yet were found to downregulate surface MHC class I at late phases. BNLF2a's expression profile and function therefore suggest it serves to protect immediate early and early proteins from immune recognition at a time when epitopes from these proteins will be maximally produced. The lack of restoration of antigen presentation by BNLF2a-deleted targets during late virus replication suggests BNLF2a is one of several immune evasion proteins encoded by this virus.

53 (RegID: 1276; 1277; 1278)
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NEWLY SYNTHESISED POLYPEPTIDES VERSUS MATURE PROTEIN AS ENDOGENOUS ANTIGEN SOURCES FOR CD4+ AND CD8+ T CELL RECOGNITION : STUDIES WITH EBNA1 AND EBNA3B

Mackay L.K.1, Long H.M.1, Brooks J.M.1, Mautner J.2 & Rickinson A.B1. 1CRUK

Oralabstract:
EBV-transformed lymphoblastoid cell lines (LCLs) are directly recognised by CD8+, and in some cases also by CD4+, T cells specific for EBV latent cycle proteins. It is not clear to what extent the peptide epitopes mediating such recognition derive from newly synthesised polypeptides degraded shortly after translation, or from turnover of the intracellular pool of mature protein. To address this, we have generated epitope-negative LCLs (B cells transformed with EBNA3B-knockout virus or with a virus strain carrying a variant EBNA1 allele) and introduced the B95.8 EBNA3B and EBNA1/E1ΔGA coding sequences under a Dox-regulated promoter that gives firm temporal control over antigen expression. The kinetics of new antigen synthesis following addition of the drug, and of antigen degradation following drug removal, were followed by immunoblotting. In parallel the same cells were assayed as targets for recognition by CD8+ and CD4+ T cell clones specific for defined EBNA3B and EBNA1 epitopes. For both antigens, CD8+ T cell recognition was first detectable within 6hr of antigen induction, long before the mature protein reached physiologic levels, and rose to a plateau within 24hr; CD4+ T cell recognition was not detected until after 24hr and rose slowly over 7 days to its plateau level. On switching off new antigen synthesis, CD8+ T cell recognition fell rapidly, at rates consistent with the half-life of pre-existing MHC-peptide complexes on the cell surface, and became undetectable despite the continued presence of a large pool of mature protein within the target cells. By contrast, CD4+ T cell recognition declined much more slowly, mirroring the half-life of the mature protein detectable by immunoblotting. We infer that, in LCL cells, newly-synthesised polypeptides are the major source of EBV-derived epitopes presented to CD8+ T cells, whereas the mature protein pool is the major source of antigen processed intracellularly for presentation to CD4+ T cells.

NOTES:

Oral speaker
Abstract

Session 11: DIGNOSTICS, DRUG DEVELOPMENT AND THERAPEUTICS

Chairpersons: Stephen Gottschalk, Kwok-Wai Lo

Monday, November 10, 14:40-15:40

54 (RegID: 1139)
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IMAGING EBV-ASSOCIATED NASOPHARYNGEAL CARCINOMA IN VIVO

:De-Xue Fu, Yvette Tanhehco, Jianmeng Chen, James Fox, Catherine Foss, Sridhar Nimmadda, George Sgouros, Martin Pomper and Richard Ambinder

Oralabstract:

Epstein-Barr virus (EBV) has been identified in a wide variety of lymphomas and carcinomas. The virus encodes kinases that phosphorylate nucleoside analogs such as 2'-deoxy-2'-fluoro-5-iodo-1-beta-D-arabinofuranosyluracil (FIAU). We hypothesized that it might be possible to use the viral enzyme to concentrate [125I]FIAU specifically within tumor cells harboring virus and thus deliver imaging and therapeutic radiation. In previous study, we found that bortezomib (Velcade) is a potent stimulator of viral kinase expression in EBV (+) lymphoma cell lines in vitro and EBV-associated lymphoma can be imaged by induction of viral gene expression with bortezomib treatment in vivo (Fu. D., et al. Clin Cancer Res 13, 1453-1458, 2007). Moreover a therapeutic effect was demonstrated with [131I]FIAU in xenografts derived from EBV (+) human lymphoma and gastric cancer cell lines as well as KSHV (+) primary effusion lymphoma (Fu. D., et al, Nature Medicine, 2008 Sep 7 [Epub ahead of print]). In this study, we extend those results to targeting of EBV-associated nasopharyngeal carcinoma (NPC). Ex vivo bio-distribution studies with [125I]FIAU showed that uptake and retention of [125I] FIAU is highly specific for NPC cells that are EBV positive upon lytic infection but not when virus is in the latent state. Planar gamma imaging and SPECT/CT imaging with [125I]FIAU of NPC-bearing SCID mice showed selective concentration of radiotracer in tumor tissue in EBV-associated NPC when animals were pretreated with the bortezomib. We found dramatically increased [125I] FIAU uptake within EBV (+) NPC at 72 hours after injection of [125I]FIAU. These results indicate that treatment with bortezomib leads to selective concentration of radiolabeled FIAU in the EBV-associated NPC xenografts and may provide a simple way for the localization, monitoring and therapy of EBV-associated NPC that could be translated to the clinic.

55 (RegID: 1290)
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HUMORAL IMMUNE RESPONSES TO EPSTEIN-BARR VIRUS ENCODED TUMOR ASSOCIATED PROTEINS AND THEIR PUTATIVE EXTRACELLULAR DOMAINS IN SERA FROM NASOPHARYNGEAL CARCINOMA PATIENTS AND REGIONAL CONTROLS.

Dewi K. Paramita, Christien Fatmawati, Hedy Juwana, Frank G. van Schaijk, Jajah Fachiroh, Sofia M. Haryana, and Jaap M. Middeldorp.

Oralabstract:

In nasopharyngeal carcinoma (NPC) Epstein-Barr virus (EBV) latency type-II proteins EBNA1, LMP1, LMP-2A/-2B and BARF1 are expressed. In a large panel of NPC patients (n=151) and regional controls (n=92) we analysed IgG and IgA antibody responses to these non-self tumor-associated antigens by three different approaches, i.e. indirect immunofluorescence assay (IFA) and immunoblot (IB) using baculovirus-expressed recombinant proteins and Enzyme Linked Immunosorbent Assay (ELISA) using distinct synthetic peptide epitopes, in particular focusing on the putative extracellular domains.

Compared to the abundant VCA-/EAd-IgG/-IgA responses in the same patients, only low levels of antibodies to LMP1, LMP2A and BARF1 could be detected in NPC sera. Using IFA, 59.4%, 69.6%, 68.8% of NPC patients had only low titered (1:10 - 1:40) IgG to LMP1, LMP2A and BARF1 respectively, while IB showed 24.2%, 12.5% and 12.5% at 1:10 serum dilution, respectively. IgG responses to EBNA1 had high titer (>1:200) in 100% and 94.9% of NPC patients using IFA and IB, respectively. Only few IgA responses were detected in NPC sera against these proteins, except for IgA-EBNA1 (81.8% using IFA and 56.5% using IB). Healthy EBV carriers from the same region were virtually devoid of any antibody response to these EBV tumor-associated antigens, except for IgG to EBNA1. ELISA using synthetic peptides-derived from different intracellular and putative extracellular domains of LMP1, LMP2A and BARF1 also yielded low antibody responses (IgG and IgA) in NPC patients. Fine mapping revealed that, –when existing-, most responses consist of IgG to intracellular C-terminus of LMP1 (62.9%).

Immunization of rabbits with synthetic peptides used in ELISA generated specific antibody responses that served as positive control. Importantly, rabbit antibodies against putative LMP1 and LMP2 extracellular domains were shown to specifically stain extracellular domains of LMP1 and LMP2 on viable EBV transformed cells and were able to mediate complement-driven cytolysis on 50.4% and 59.4% of X50/7 and 35.0% and 35.9% of Raji cell lines by anti-LMP1 loop-1 and -3 antibodies respectively, and nearly 22% of both cell lines either by anti-LMP2 loop-2 or -5 antibodies.

Data generated in this study demonstrate that EBV-encoded tumor-associated antigens are only marginally immunogenic for humoral immune responses in NPC patients. However, specific stimulation using exogenous peptide constructs may enhance the generation of such antibodies which can mediate the killing activity through antibody dependent cytotoxicity. This opens options for peptide-based tumor vaccination in patients carrying EBV latency-II type tumors such as NPC.

56 (RegID: 1762)
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HSP90 INHIBITORS INDUCE KILLING OF EPSTEIN-BARR VIRUS-POSITIVE TUMOR CELLS AND REDUCE EXPRESSION OF EBNA1

Xiaoping Sun, Shidong Ma, Elizabeth Barlow, Shannon Kenney

Oralabstract:

The requirement of EBNA1 for EBV genome replication, partition, and transcription, along with its possible direct contribution to tumorigenesis, suggests that EBNA1 could serve as a target for developing novel strategies to treat EBV-positive tumors. Hsp90 is a chaperone protein that has a restricted number of client proteins in normal cells but is over-expressed in, and appears to be required for proliferation and survival of, some types of tumor cells. Here we demonstrate that Hsp90 inhibitors, including geldanomycin, 17-AAG, and 17-DMAG, inhibit EBNA1 expression in various epithelial and lymphoid cells latently infected with EBV. In transfection experiments, Hsp90 inhibitors reduce the expression of EBNA1, but not LMP1, in the context of the SG5 vector, and this effect requires the presence of the EBNA1 Gly-Ala repeat domain in some cell types. Geldanomycin inhibits in vitro translation of EBNA1, but not BZLF1, in reticulocyte lyates and this effect requires the Gly-Ala repeat domain. However, proteasomal inhibitors do not reduce the effect of Hsp90 inhibitors on either endogenous EBNA1 or transiently expressed EBNA1. These results suggest that efficient translation of EBNA1 requires HSP90. Treatment with Hsp90 inhibitors using low doses previously shown to be nontoxic in patients resulted in essentially 100% cell death in established lymphoblastoid cell lines (LCLs) as well as in primary B cells infected with EBV for only a few weeks. In contrast, these doses of Hsp90 inhibitors only slightly decreased growth/viability of telomerase-immortalized keratinocytes and uninfected normal primary B cells. Most importantly, the ability of LCLs to induce lymphoproliferative disease in SCID mice was strongly inhibited using a low dose of 17-AAG. These results suggest that EBV-dependent cell killing may be at least partially caused by the decreased expression of EBNA1 and that inhibition of Hsp90 may be a particularly effective method for treating EBV-induced lymphoproliferative disease.

NOTES:

Poster Sessions

Session 1: Latent Infection

57 (RegID: 1087)

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EPSTEIN-BARR VIRUS BART GENE EXPRESSION

Maha Al-Mozaini, Gustavo Bodelon, Claudio Elgueta Karstegl, Boquan Jin, Mohammed Al-Ahdal and Paul J. Farrell.

Posterabstract：

Introns from the EBV BART RNAs produce up to 20 miRNAs but the spliced exons of the BART RNAs have also been investigated as possible mRNAs, with the potential to express the RPMS1 and A73 proteins. Recombinant RPMS1 and A73 proteins were expressed in E. coli and used to make new monoclonal antibodies that reacted specifically with artificially expressed RPMS1 and A73. These antibodies did not detect endogenous expression of A73 and RPMS1 proteins in a panel of EBV infected cell lines representing the different known types of EBV infection. Also BART RNA could not be detected on Northern blots of cytoplasmic polyA+ RNA from the C666.1 NPC cell line, arguing against an mRNA role for BART RNAs. In contrast, some early lytic cycle EBV mRNAs were found to be expressed in C666.1 cells. Artificially expressed A73 protein was known to be able to bind to the cellular RACK1 protein and has now also been shown to be able to regulate calcium flux, presumably via RACK1. Overall, the results support the conclusion that the miRNAs are the functionally important products of BART transcription in the cell lines studied because the A73 and RPMS1 proteins could not be detected in natural EBV infections. However, the possibility remains that A73 and RPMS1 might be expressed in some situation because of the clear potential relevance of their biochemical functions.

58 (RegID: 1093; 1094; 1095)
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INVOLVEMENT OF HSC70 AND EBV LATENT MEMBRANE PROTEIN-1 IN THE REGULATION OF CELL MITOSIS

Robert Qi

Posterabstract：

Although the latent membrane protein-1 (LMP1) of the Epstein-Barr virus (EBV) is believed to be important for tumorigenesis, the precise contribution of this viral oncoprotein is poorly understood. We have found that LMP1 interacts with Hsc70 (heat shock cognate 70 kDa protein), an Hsp70 family member that performs various functions related to cell proliferation and tumorigenesis. Hsc70 expression has been shown to correlate with EBV infection. In this study, we show that Hsc70 displays cell cycle-dependent centrosomal localization. During mitosis, the centrosomal level of Hsc70 increases dramatically from prometaphase to metaphase and decreases abruptly after metaphase. These observations suggest a mitotic function of the protein at centrosomes. Suppression of Hsc70 expression by RNA interference caused defects in the formation of mitotic spindles and in chromosomal alignment to the metaphase plate, pointing to an important role of Hsc70 for genome stability during cell division. The mitotic functions of Hsc70 and LMP1 were further characterized using time-lapse microscopy. Our results suggested that LMP1 might have a coordinated mitotic function with Hsc70 during EBV-mediated tumorigenesis.

59 (RegID: 1107 ; 1108)

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A SYSTEM BIOLOGY APPROACH FOR THE ROLE OF EBNA3C IN MODULATING THE S-G2 JUNCTION OF CELL CYCLE.

Tathagata Choudhuri

Posterabstract：

The Epstein-Barr virus (EBV) infects most of the human population and persists in B-lymphocytes for the lifetime of the host. During the establishment of latent infection a unique repertoire of genes are expressed. One of those genes, the EBV nuclear antigen EBNA3C is essential for growth transformation of primary B-lymphocytes in vitro and regulates the transcription of a number of viral and cellular genes important for the immortalization process. Previous work of mine demonstrates an associated function of EBNA3C, which involves disruption of the G2/M checkpoint. Those results indicated the existence of that EBNA3C could regulate the G2/M component of the host cell cycle machinery. Considering these, a complete understanding of the mechanism(s) and molecular targets of EBNA3C in the regulation of host cell cycle machinery is utmost necessary. We isolate enriched populations of S and G2/M populations of EBNA3C stable BJAB cell lines cells along with BJAB cells as control. In these cell populations we actually saw the specific interaction of EBNA3C in the S-G2 transition of the cell cycle. We compare both the cell line in native gel line and analyzed them in MALDI-TOF. We get about 80 different proteins which are associated with cell cycle machinery. We fit this data in a mathematical model. This model, using system biology approach tells us how EBNA3C help in the cell cycle modulation and maintaining the type III latency. Thus, this work will leads to a discovery which is not only important in adding to our understanding of the modulation of host machinery system of the host by Epstein-Barr virus nuclear antigen 3C.

60 (RegID:1115; 1116)

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PRIMA-1MET INDUCES NUCLEOLAR TRANSLOCATION OF EPSTEIN BARR VIRUS-ENCODED EBNA-5 PROTEIN

György Stuber1, Emilie Flaberg1, 2, 3, Gabor Petranyi1, 2. 5., Rita 謙 v 鰪 1, Nina R 鰊 aeus4, Elena Kashuba1, 3, Klas G. Wiman4, George Klein1 and Laszlo Szekely1, 2, 3

Posterabstract：

The low molecular weight compound, PRIMA-1MET restores the transcriptional transactivation function of certain p53 mutants in tumor cells. We have previously shown that PRIMA-1MET induces nucleolar translocation of p53, PML, CBP and Hsp70. The Epstein-Barr virus encoded, latency associated antigen EBNA-5 (also known as EBNA-LP) is required for the efficient transformation of human B lymphocytes by EBV. EBNA-5 associates with p53-hMDM2-p14ARF complexes. EBNA-5 is a nuclear protein that translocates to the nucleolus upon heat shock or inhibition of proteasomes along with p53, hMDM2, Hsp70, PML and proteasome subunits. Here we show that PRIMA-1MET induces the nucleolar translocation of EBNA-5 in EBV transformed B lymphoblasts and in transfected tumor cells. The PRIMA-1MET induced translocation of EBNA-5 is not dependent on the presence of mutant p53. It also occurs in p53 null cells or in cells that express wild type p53. Both the native and the EGFP or DSRed conjugated EBNA-5 respond to PRIMA-1MET treatment in the same way. Image analysis of DSRed-EBNA-5 expressing cells, using confocal fluorescence time-lapse microscopy showed that the nucleolar translocation requires several hours to complete. FRAP (fluorescence recovery after photobleaching) and FLIP (fluorescence loss in photobleaching) measurements on live cells showed that the nucleolar translocation was accompanied by the formation of EBNA-5 aggregates. The process is reversible since the aggregates are dissolved upon removal of PRIMA-1MET. Our results suggest that mutant p53 is not the sole target of PRIMA-1MET. We propose that PRIMA-1MET may reversibly inhibit cellular chaperons that prevent the aggregation of misfolded proteins, and that EBNA-5 may serve as a surrogate drug target for elucidating the precise molecular action of PRIMA-1MET.

61 (RegID: 1117)
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CHANGES IN THE CHEMOKINE AND CHEMOKINE RECEPTOR EXPRESSION AFTER EPSTEIN-BARR VIRUS INFECTION OF TONSILLAR B CELLS

Barbro Ehlin-Henriksson1,,Liang Wu1, Alberto Cagigi1, Frida Mowafi1, George Klein1 and Anna Nilsson1,2

Posterabstract:
The primary Epstein-Barr virus (EBV) infection occurs in the oropharynx, where the virus infects B cells and subsequently establishes latency in the memory B cell compartment. We have previously shown that EBV modulates the expression of CXCR4 and reduces migration to CXCL12, ligand of CXCR4,

We show that EBV infection in vitro reduces the expression of CXCR5 and CCR7 on tonsil B cells by day 7 after infection. Furthermore, EBV infection affects the chemotactic response already after 2 days with a reduction of both the CXCL13- and CCL21-induced migration.

Using gene expression profiling, we identified an additional set of chemokines and chemokine receptors that were changed upon EBV infection in comparison to non-infected tonsillar B cells. In particular, mRNA expression for CCR9 and the complement receptor C5AR1 (CD88) was increased.

CXCL13 is produced in B cell follicles whereas CCL21 is expressed in the T cell zone. It may be speculated that changing the response of the EBV infected B cells to CXCL13 and CCL21 may serve the viral strategy by permitting the infected cells to reside in the extrafollicular areas, rather than to be directed to the B cell follicle and subsequently to the B-T cell border. CCR9 and CD88 are both involved in mucosal homing and may participate in the positioning of the EBV-infected B cell in the extrafollicular area.

62 (RegID: 1206)
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INTERFERON REGULATORY FACTORS 4 IS INVOLVED IN EPSTEIN-BARR VIRUS-MEDIATED TRANSFORMATION OF HUMAN B LYMPHOCYTES

Dongsheng Xu 1, Lingjun Zhao 2, Luis Del Valle3, Judith Miklossy3, and Luwen Zhang 1

Posterabstract:

Epstein-Barr virus (EBV) infection is associated with many human malignancies. In vitro, EBV transforms primary B cells into continuously growing lymphoblastoid cell lines. The EBV latent membrane protein 1 (LMP-1) is required for EBV transformation processes. Interferon regulatory factor 4 (IRF-4) has oncogenic potential. We find that high levels of IRF-4 are associated with EBV transformation of human primary B cells in vitro, and with EBV type III latency in which LMP-1 is expressed. EBV LMP-1 stimulates IRF-4 expression in B lymphocytes. The signaling from LMP-1 is required and cellular NF-κB is involved in the stimulation of IRF-4 by LMP-1. Growth of EBV-transformed cells is inhibited when IRF-4 is specifically down-regulated. We further show that IRF-4-knockdown cells have lower proliferation but higher apoptotic rates than control cells. Finally, IRF-4 is expressed in significant numbers of specimens of primary central nervous system (CNS) lymphomas (12/27; 44.4%), an EBV associated malignancy. The association between the expression levels of LMP-1 and IRF-4 is statistically significant ($p=0.011$) in these CNS lymphoma specimens. Our data suggest that IRF-4 may be a critical factor in EBV transformation and a useful target in the therapy of EBV-mediated neoplasia.

63 (RegID: 1236)
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IDENTIFICATION OF DELTANP63 AS A MODULATOR OF LMP1 EXPRESSION IN EBV-INFECTED EPITHELIAL CELLS

J.Jia, C.W. Dawson and L.S.Young

Posterabstract:
Epstein–Barr virus (EBV) is a ubiquitous human herpesvirus implicated in the development of both lymphoid and epithelial tumors. Whereas expression of the LMP1 protein is variable in NPC, LMP2A is more frequently expressed. Previous studies from our group have shown that epithelial cell lines infected with an LMP2A-deleted EBV (LMP2A-rEBV) express low levels of LMP1, suggesting that LMP2A negatively regulates LMP1 expression in epithelial cells (Stewart, 2004). We now show that LMP1 is subject to regulation by the p53 transcription factor family member deltaNp63, and that LMP2A regulates LMP1 expression by targeting deltaNp63 for degradation.

In this study we show that LMP1 protein is elevated in LMP2A-rEBV infected carcinoma cells expressing high levels of endogenous deltaNp63. Moreover, exogenous expression of deltaNp63 could further increase LMP1 promoter activity and protein expression, whereas silencing deltaNp63 by RNAi reduced LMP1 promoter activity and decreased LMP1 protein.

A role for LMP2A in this process was established, as transient expression of LMP2A into deltaNp63-positive carcinoma cells stably infected with LMP2A-rEBV, resulted in a marked reduction in deltaNp63 expression and a corresponding decrease in LMP1 protein. ChIP assay confirmed that deltaNp63 bound to a region located upstream of the L1-TR LMP1 promoter. This study identifies deltaNp63 as a key effector molecule in modulating the expression of LMP1 in EBV-infected epithelial cells.

64 (RegID: 1259)
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EBNA-LP RELOCALIZES SMRT/NCOR AND HDAC5 FROM MAD BODIES TO THE NUCLEOPLASM

DANIEL PORTAL AND ELLIOTT KIEFF

Posterabstract:
Epstein-Barr nuclear antigen (EBNA) leader protein (EBNA-LP) coactivates promoters with EBNA2 and is important for Epstein-Barr virus immortalization of B cells. EBNA2 activates transcription by associating with RBP/CSL and displacing SMRT/NCoR-HDAC5 corepressor complex. We investigated EBNA-LP's role in coactivation with EBNA2 by affecting SMRT/NCoR-HDAC5 from their subnuclear localization. At steady state, SMRT/NCoR-HDAC5 localize to Matrix-Associated Deacetylase (MAD) Bodies as well as diffusely in the nucleus and cytoplasm. EBNA-LP's presence completely relocalizes SMRT/NCoR and HDAC5 from MAD bodies to a diffuse nuclear distribution. Moreover, SMRT/NCoR and HDAC5's diffuse distribution completely colocalizes with EBNA-LP's distribution, but not with active transcription as determined by H3K4 co-immunostaining, suggesting that EBNA-LP is not present at sites of active transcription. MAD bodies' disruption appears to be dependent on EBNA-LP's ability to interact with HA95, a nuclear matrix protein, since an EBNA-LP protein unable to associate with HA95 is 10 fold less efficient at relocating SMRT/NCoR and HDAC5.These data further strengthen EBNA-LP's role in coactivation of transcription by relocating repressors from their steady state subcellular localization.

65 (RegID: 1320)
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AN IN VITRO MODEL FOR LATENCY IN THE GAMMAHERPESVIRUSES

K. Mutyambizi (1, 2), H. Coleman (1), V. Connor (1), S. Efstathiou (1).

Posterabstract:
Background: The human gammaherpesviruses EBV and KSHV realize their oncogenic potential during latent infection. The species specificity of EBV and KSHV has hindered their study in animal models. Murine gammaherpesvirus (MHV-68) efficiently infects the laboratory mouse providing a representative gammaherpesvirus for the study of latency. Existing models for latency in MHV-68 have been confounded by spontaneous reactivation. MHV68 ORF50, homologous to BRLF1 in EBV, is the major MHV68 viral trans-activator mediating the switch between lytic and latent infection. To facilitate studies on viral oncogenesis in gammaherpesvirus infection, an in vitro model of latency was established using replication defective mutants (Δ50 MHV68) with the GFP reporter gene under ORF50 promoter control.
Methods: 3T3 fibroblasts, RAW macrophages and NS0 B cells were coordinately infected with Δ50 MHV-68 and screened for spontaneous reactivation using flow cytometry. Infected cultures were artificially reactivated from latency by super-infection with WT MHV-68 to determine reactivation efficiencies. Expression profiles of latent and lytic transcripts were established by quantitative real time RT-PCR over a 3 day and 30 day time course to establish the transcriptional profile in early latency and during maintenance of latency.
Results: Latency was readily established and maintained in 3T3 and RAW cells, with reactivation efficiencies of 40-60%. The resistance to infection of NS0 cells by Δ50 MHV-68 restricted their further study. Latently infected 3T3 and RAW cultures exhibited stable transcription of latency associated genes including ORF73 indicating episomal maintenance. Lytic transcripts were negligibly transcribed with no spontaneous reactivation detected.
Conclusion: We present a clean latency system devoid of contaminating lytic competent virus. It is a tractable system enabling analysis during the establishment and maintenance phases of latency. Furthermore, we distinguish the latent transcriptional profile from the lytic profile during early infection. In this system, latent virus can be artificially reactivated, allowing diverse applications.

66 (RegID: 1395)
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EBV INDUCES AN ATM AND CHK2-DEPENDENT DNA DAMAGE RESPONSIVE PATHWAY EARLY AFTER INFECTION THAT LIMITS B CELL PROLIFERATION AND LONG-TERM LCL OUTGROWTH

Micah Luftig, Eleonora Forte, Olena Rusyn, Pavel Nikitin, Chris Yan, David Tainter, Jing Guo

Posterabstract:
Epstein-Barr virus infection of primary B cells leads to long-term outgrowth of less than 10% of infected cells. As such, a robust innate tumor suppressor response likely exists in more than 90% of infected cells preventing outgrowth. Recently, the DNA damage response (DDR) to oncogene-induced replicative stress has been shown to suppress proliferation acting as an early barrier to tumorigenesis through inducing senescence and apoptosis. We therefore asked whether EBV infection of primary B cells activated the DNA damage response and what the functional consequence of this response was on long-term LCL outgrowth. We found that within two days of infection, EBV induced a robust DDR as determined by phosphorylation of key checkpoint kinases ATM and Chk2, the ATM-specific phosphorylation of p53, and the accumulation of DDR components, including 53BP1, to intra-nuclear foci. These activations were dependent on latency III gene expression as P3HR1 and UV-inactivated B95-8 infected cells did not induce these events. Importantly, inhibition of the DNA damage sensing kinase ATM or its downstream effector Chk2 led to a 5-10 fold increase in EBV transformation efficiency as well as the increased proliferation of B cells within the first two weeks following infection. Thus, EBV latency III gene expression, likely through the induction of Myc activity early after infection, upregulates a DNA damage responsive pathway that limits transformation. The significance of these activations in the context of oncogenic stress and DNA damage pathways will be discussed.

67 (RegID: 1459)
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BZLF1-KNOCKOUT EBV TRANSFORMS PRIMARY B-LYMPHOCYTES AS EFFICIENTLY AS WILD-TYPE EBV

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Posterabstract:
BZLF1 gene of EBV encodes a transactivator that triggers the onset of EBV lytic infection. Previously, we reported that BZLF1 was expressed in B-lymphocytes during early phase of infection, but its biological significance was unclear. So, we have examined whether BZLF1 contributes to B cell transformation in this study. We generated BZLF1-knockout (KO) EBV to clarify the issue. Homologous recombination in Akata cells was used to obtain BZLF1-KO EBV. Akata cells harboring BZLF1-KO EBV were not induced to enter the lytic cycle by treatment of anti-human IgG or chemical inducers. The cells entered the lytic cycle only when BZLF1 gene was expressed in trans, but the amount of produced virus was quite limited. We then utilized AGS cells as alternative virus-producing cells. AGS cells harboring wild type EBV spontaneously entered the lytic cycle, while cells harboring BZLF1-KO EBV did not. When BZLF1 gene was expressed in trans, AGS cells harboring BZLF1-KO EBV entered the lytic cycle and produced a large amount of virus. Wild type EBV but not BZLF1-KO EBV caused BZLF1 expression after infection to primary blood mononuclear cells. Quantitative transformation assay in vitro revealed that BZLF1-KO EBV could transform B-lymphocytes as efficiently as wild type EBV. The LCLs established by BZLF1-KO EBV were indistinguishable from those established by wild type EBV including virus copy number and viral latent gene expression. These results indicate that EBV can transform primary B cells efficiently regardless of BZLF1 expression or lytic infection.

68 (RegID: 1517; 1675)

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EBNA-1 PROMOTES GENOMIC INSTABILITY BY INDUCING THE PRODUCTION OF ROS

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Posterabstract:

The Epstein-Barr virus (EBV) nuclear antigen (EBNA)-1 is the only viral protein expressed in all EBV carrying malignancies but its contribution to oncogenesis has remained enigmatic. We show that EBNA-1 induces chromosomal aberrations, DNA double-strand breaks and engagement of the DNA damage response (DDR). These signs of genomic instability are associated with the production of reactive oxygen species (ROS) and are reversed by antioxidants. The catalytic subunit of the NADPH oxidase, NOX2/gp91phox, is transcriptionally activated by EBNA-1, while inactivation of the enzyme by chemicals or RNAi halts ROS production and DDR. These findings highlight a novel function of EBNA-1 that explains its capacity to promote B-cell immortalization and malignant transformation.

69 (RegID: 1676; 1678; 1679)

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THE EPSTEIN-BARR VIRUS ONCOPROTEIN, LATENT MEMBRANE PROTEIN-1 REPROGRAMS TONSILLAR B CELLS TOWARDS A HODGKIN'S REED STERNBERG LIKE PHENOTYPE

Martina Vockerodt,1 Katerina Vrzalikova1,2, Susan L Morgan,1 Wenbin Wei,1 Dieter Kube,3 Lawrence S Young, 1 Ciaran B Woodman,1 and Paul G Murray1
1 School of Cancer Sciences, University of Birmingham, UK, 2 Laboratory of Molecular Pathology & Department of Pathology, Faculty of Medicine, Palacky University, Olomouc, Czech Republic, 3 Zentrum Innere Medizin, Abteilung Hämatologie und Onkologie, Georg-August-Universität Göttingen, Germany

Posterabstract：

Although the transforming capacities of the Epstein-Barr virus latent membrane protein-1 (LMP1) are well established, its precise role in the development of EBV-associated lymphoma is poorly defined. As a first step to better understand how LMP1 contributes to the early stages of B cell transformation, we developed a method for the efficient expression of LMP1, and for the analysis of LMP1-induced signaling pathways, in different subtypes of normal human tonsillar B cells. When expressed in germinal centre (GC) B cells, LMP1 induced one-quarter of the transcriptional changes characteristic of Hodgkin's lymphomas. These changes included the striking down-regulation of B cell differentiation markers and of components of B cell receptor (BCR) signaling such as CD79A, CD79B, CD19, CD20 and BLNK, and the up-regulation of several anti-apoptotic genes (BCL2, CFLAR) and immunomodulatory molecules (CCL17, LTA). Furthermore, LMP1 modulated the expression of B cell specific transcriptional regulators, such as ID2, IRF4, MYC, EBF, SOX4 and PAX5. Importantly, the down-regulation of BCR signaling molecules was also observed in LMP1-expressing naïve B cells, indicating that LMP1 might drive the differentiation of these B cells towards a post- GC phenotype. Our results suggest that as well as providing a direct anti-apoptotic signal, LMP1 might also contribute to B cell transformation by bypassing critical checkpoints in B cell development.

70 (RegID: 1704; 1724; 1726; 1876)

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EBV LMP2A MODULATES THE TYROSINE KINASE SYK TO INDUCE INVASION IN EPITHELIAL CELLS

Chen F, Gish G1, Ingham RJ1,2, Werner M, Pawson T1,3, Ernberg I*

Posterabstract:

Epstein-Barr virus (EBV) is associated with the epithelial cell malignancy nasopharyngeal carcinoma (NPC), which frequently express the Latent Membrane Protein 2A (LMP2A). Human epithelial cells engineered to express LMP2A generate tumours when transplanted into SCID mice, suggesting a role for LMP2A in tumourigenesis. Using mass spectrometric analysis, we found the spleen tyrosine kinase (Syk), as an epithelial cellular protein that interacts with the phosphorylated ITAM-motif found in LMP2A. Introduction of LMP2A into the human epithelial cell lines TWO3 and 5637, induced constitutive tyrosine phosphorylation of Syk. Expression of LMP2A dramatically enhanced the invasive capacity of epithelial cells, depending on Syk recruitment. Downregulation of Syk by shRNA also increased epithelial cell invasion. Thus Syk may normally suppress epithelial cell motility, and LMP2A redirects Syk signalling to promote invasion. However, in a Syk negative cell line HeLa, LMP2A was not able to induce the invasive capacity of these cells. Furthermore, Syk is able to bind to a6b4 integrin subunit in a tyrosine phosphorylation independent manner by immunoprecipitation in a cell line 5637. There are piles of evidences of the role of beta 4 integrin in carcinoma cell migration and invasion. This provides one link between Syk and invasive capacity. These data posit a role for Syk in epithelial cell biology that is co-opted by LMP2A.

71 (RegID: 1715; 1717; 1722)

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EBNA 2 STIMULATES TRANSCRIPTIONAL ELONGATION THROUGH PTEFB AND SPT5

Richard Palermo, Helen Webb and Michelle J. West

Posterabstract:

EBNA 2 is essential for EBV-driven transformation and for the continuous proliferation of infected cells. EBNA 2 and EBNA-leader protein transcripts are expressed early infection from the EBV W promoter. EBNA 2 then mediates activation of the main latency promoter, Cp, resulting in promoter switching and the transcription of long (approximately 120kb) transcripts that encode the remaining EBNAs required for immortalisation.

We have previously shown that EBNA 2-activated transcription requires the activity of the kinase subunit (CDK9) of the elongation factor pTEFb, promoting phosphorylation of the C-terminal domain (CTD) of RNA polymerase II on serine 5 during Cp transcription.

We now demonstrate that both the CDK9 and cyclin T1 subunits of pTEFb are recruited to the C promoter in the presence of EBNA 2 stimulating large increases in both serine 2 and 5 phosphorylation on the pol II CTD at promoter distal regions of the viral genome that are dramatically reduced in the presence of the CDK9 inhibitor DRB. In addition to increased Pol II retention and phosphorylation, we also show that EBNA 2 recruits the elongation factors, DRB sensitivity inducing factor (DSIF) and Negative elongation factor (NELF), to Cp. DRB treatment also increases the association of EBNA 2 and pTEFb with Cp in a novel mechanism. Together these data provide evidence that EBNA 2 promotes elongation to distal regions of the template and suggest that the promotion of transcriptional elongation may be the driving force behind promoter switching during early EBV infection.

72 (RegID: 1886)

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FINDING THE REGULATORS OF EPSTEIN-BARR VIRUS LATENCY

Omid R. Faridani, Annette Dirac, Christian Gentili, Rene Bernards, Maria G. Masucci

Posterabstract:

Epstein–Barr virus (EBV) is associated with a variety of lymphomas and carcinomas. In the related cancers, EBV remains latent in cells to avoid immune surveillance. One potential approach would involve modulating genes that regulate latency maintenance to reactivate latent virus in cancer cells into lytic replication causing cells to become targets for immune system. In this study, we used RNAi screening of Akata-Bx1 to find genes that are regulators of latency/lytic shift.

NOTES:

Poster Sessions

Session 2： Viral Replication

73 (RegID: 1025; 1026)

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CRYSTAL STRUCTURE OF EBV DNASE

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Posterabstract：

The BGLF5 protein of EBV, also called DNAse or alcaline exonuclease is one of the enzymes needed for the replication of viral DNA which are conserved throughout the herpesvirus family. Still, hardly any structural information was available for this important enzyme of the lytic cycle. It is likely to be involved in recombination and the processing of branched replication intermediates occurring during the rolling circle replication of the viral DNA due to its 5'-3' exonuclease and additional endonucleic activity active against DNA. DNAse defective mutants in herpes simplex virus, where the enzyme has been most studied, lead to inefficient replication and packaging and drastically reduced titers of infectious virus (Martinez *et al.*, 1996). Recently an additional host shutoff function, most likely through an increased mRNA degradation has been described (Rowe *et al.*, 2007) for the EBV protein. EBV DNAse, an enzyme of 470 aa has been expressed in insect cells using baculovirus and its structure has been solved to 3 Å resolution by x-ray crystallography. It shows a monomeric structure where the central domain resembles the the trimeric bacteriophage λ exonuclease, a much shorter (226 aa) but trimeric enzyme. An additional N-terminal domain and a 100 aa insertion forming a domain which carries the nuclear localization signals account for the extra residues of herpesvirus DNAses. Using the distantly related structure of the restriction endonuclease HincII, the interaction with DNA could be modelled in order to understand better substrate specificity and the enzyme's processivity. Currently, these hypotheses are being validated by additional biochemical experiments.

74 (RegID: 1084)
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REGULATION OF EPSTEIN-BARR VIRUS BZLF1

Carol McDonald and Paul J. Farrell

Posterabstract:
BZLF1 is the immediate early gene in Epstein-Barr virus (EBV) that mediates the switch between latency and the lytic cycle in response to BCR signal transduction. The BZLF1 gene is under the control of the Zp promoter.

Mutagenesis of the Zp promoter using a luciferase reporter assay allowed quantitative studies of the promoter in EBV infected cells. Eight new Zp mutants were constructed to investigate Zp sequences that had not previously been tested for their contribution to promoter activity in response to anti-IgG induction. The ZID MEF2 binding site, previously identified by DNAse footprinting, was for the first time demonstrated to be functionally important during the early stages of Zp activation. We have also found that splicing of the endogenous XBP-1 transcription factor is activated by BCR signal transduction with the same time course as the early promoter induction events, bringing together molecular analysis of the Zp promoter with current in vivo models for EBV reactivation.

Part of the BZLF1 protein structure is related the b-ZIP family of proteins. The dimerisation domain of BZLF1 consists of an alpha-helical coiled coil structure which breaks at a proline residue, forming a C-terminal (CT) tail structure that runs anti-parallel to the zipper (Petosa et al, 2006 Molecular Cell 21:565-572). BZLF1 has dual functions during the lytic cycle. It acts as a transcriptional activator but later initiates viral replication by binding to the origin of lytic replication. Many novel mutations in the zipper and tail region were investigated to determine the importance of the CT tail binding to the zipper region in transactivation and replication. The results indicate that binding of the CT tail to the zipper region is required for lytic cycle DNA replication but not for promoter transactivation by BZLF1.

75 (RegID: 1110)
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EBV'S ABORTIVE LYTIC PHASE IN PRIMARY B CELLS

Markus Kalla, Anne Schmeinck, Martin Bergbauer, Dagmar Pich, Wolfgang Hammerschmidt

Posterabstract:

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Initially, Epstein-Barr Virus (EBV) establishes a strictly latent infection in all cells it infects. Synthesis and release of progeny virus does not take place for weeks post infection. The molecular basis of this phenomenon is unclear. In latently infected primary B cells, similar to the viral Bcl-2 homologues (vBcl-2) BHRF1 and BALF1 (Altmann and Hammerschmidt, PLoS Biol 2005, 3(12):e404), the two viral lytic switch genes, BZLF1 and BRLF1, are expressed early after infection. The early expression of vBcl-2 genes is essential for the transformation of infected human B cells but the role of BZLF1 and BRLF1 early after infection is unclear. Here we concentrate on the analysis of the early phase of infection, which we termed EBV's abortive lytic phase.
With the help of a PCR-based EBV-microarray we show that several lytic genes are transcribed early after infection of primary B cells. In contrast, many transcripts of structural genes were not detected. Additional experiments showed that the BZLF1 and BRLF1 genes, which encode the viral transactivators, Zta and Rta, respectively, are detectable initially after infection of primary B cells. We constructed a recombinant EBV 'reporter', which records the expression of EBV's immediate early (IE) genes at the single cell level. Our experiments indicate that EBV's IE genes are expressed in the majority of newly infected B cells. Surprisingly, expression of both IE genes does not lead to de novo synthesis of progeny virus but results in an abortive lytic phase, only. We will provide experimental data that indicate a role for the early expression of BZLF1 during B cell infection.

76 (RegID: 1130; 1945)

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H2O2 INHIBITS BCR-DEPENDENT IMMEDIATE EARLY INDUCTION OF EBV GENES IN BURKITT'S LYMPHOMA AKATA CELLS

Helen I. Osipova-Goldberg, Lyudmila V. Turchanowa, and Josef M. Pfeilschifter

Posterabstract:

The critical step in the Epstein-Barr virus (EBV) transition from latency to lytic replication is activation of the viral immediate early (IE) genes, BZLF1 and BRLF1. Their induction in Burkitt's lymphoma Akata cells is directly targeted by the B cell receptor (BCR) signaling. On the other hand, BCR stimulation causes an outwardly directed superoxide (O2•¯) burst leading to massive generation of reactive oxygen species in the cell environment. Our goal was to investigate the role of BCR-related redox changes in the IE reactivation of EBV. Production of O2•¯ by stimulated Akata cells was measured using chemiluminescent dyes, lucigenin, MCLA and coelenterazine. Expression of EBV IE genes was analyzed by TaqMan® Real-Time PCR and Western Blot assays. Catalase activity and H2O2 concentration were evaluated using Amplex Red assays. It was found that neutralization of the O2•¯ surge by specific scavengers, Tempol and TMPyP did not affect the virus IE gene induction. On the other hand, elevation of the H2O2 concentration in Akata cell suspensions inhibited induction of the virus IE mRNA and BZLF1 protein. It was further found that Akata cells exhibit catalase-like activity that is stimulated by the BCR cross-linking. Altogether, the obtained results provide new evidence regarding distinct effects of O2•¯ and H2O2 on the BCR-mediated EBV reactivation. We hypothesize that H2O2 resulting from the O2•¯ surge may be instrumental in the inhibition of EBV reactivation, i.e. it may constitute a principal part of the virus shut-down mechanism.

77 (RegID: 1168; 1169; 1248)

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FUNCTIONAL EPSTEIN-BARR VIRUS RESERVOIR IN PLASMA CELLS DERIVED FROM INFECTED PERIPHERAL BLOOD MEMORY B CELLS

Yassine Al Tabaa,1 Edouard Tuaillon,1 Karine Bollore,1 Vincent Foulongne,1 Gael Petitjean,1 Jean-Marie Seigneurin,2 Christophe Duperray,3 Claude Desgranges,4-5 and Jean-Pierre Vendrell1,3*

Posterabstract:

Epstein-Barr virus (EBV) causes infectious mononucleosis, establishes latency in resting memory B lymphocytes, and is involved in oncogenesis through poorly understood mechanisms. The EBV-lytic cycle is initiated during plasma cell differentiation by mRNAs transcripts encoded by BZLF1 which induce the synthesis of EBV proteins such as immediate-early antigen (ZEBRA) and membrane antigen (MA). Therefore, we assessed the capacity of circulating EBV-infected B lymphocytes from healthy EBV sero-positive subjects to enter and complete the EBV-lytic cycle. Purified B lymphocytes were polyclonally stimulated and ZEBRA- or MA-secreting cells (SCs) were enumerated by ELISPOT assays. The number of ZEBRA-SCs ranged from 50 to 480/107 lymphocytes (median 80, 25-75 percentiles 70-150) and MA-SCs from 10 to 40/107 lymphocytes (median 17, 25-75 percentiles 10-20). MA-SCs represented only 7.7 to 28.6% of ZEBRA-SCs (median 15%, 25-75 percentiles 10.5-20%). This EBV functional reservoir was preferentially restricted to plasma cells derived from CD27+ IgD- memory B lymphocytes. In 9/13 subjects, EBV-DNA quantification in B cell culture supernatants gave evidence of the completion of EBV-lytic cycle. These results demonstrate that EBV-proteins such as ZEBRA and MA can be secreted by EBV-infected B lymphocytes from healthy carriers, a majority generating an abortive EBV-lytic cycle and a minority completing the cycle.

78 (RegID: 1226)

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THREE DIMENSIONAL STRUCTURE OF THE EPSTEIN-BARR VIRUS CAPSID

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Posterabstract:

The knowledge of EBV morphology is widely based on its homology with the others herpesviruses. The common properties of HSV-1, CMV and HHV8 icosahedral capsid structure is a triangulation number of T=16, 150 hexamers and 12 pentamers of the major capsid protein (VP5) and 320 triplex complexes located on each of the local three-fold axis. The triplex complex is an heterotrimers alpha beta 2 (VP19C and VP23). Here we report the 3D structure of the EBV capsid, reconstructed from electron cryomicroscopy (cryoEM) images and show its structural similarities and differences with HHV8. Viruses were produced in cells culture, concentrated and purified onto CsCl density gradient, localize by negative-stain and cryo-EM was performed by using a LaB6 FEI CM200 microscope equipped with a Gatan 626 cryoholder operated at 200 kV and 20,000x nominal magnification. The 500 best particles, selected manually from 19 Kodak SO163 films digitised at a 7 衧 sampling step on a Z/I Imaging PhotoScan, were used to calculate the 20 ?resolution. The 3D structure of the virus exhibit the T=16 triangulation number which characterize the herpesviridae family. The structure is very similar to the one of HHV8, in size (1250 ?in diameter) but also in the different capsomer organization. The hexons, pentons and the triplex capsomers are very similar in shape. There is anyway a big difference between all the known structure of herpesviruses and our structure. Around the 5-fold axis of the EBV capsid, at the expected position of the triplex protein, there is only a small density present. This is the first time that this kind of phenomena is visible in the herpesviridae family.

79 (RegID: 1240)
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SYSTEMATICALLY UNVEILING EPSTEIN - BARR VIRUS LATENT MEMBRANE PROTEINS' FUNCTION AND THEIR INTERACTIONS IN EPITHELIAL CELL BY HOMOLOGOUS RECOMBINATION TECHNOLOGY

H. Qin, J.D. O'Neil, C.W. Dawson, O. Tolmachov and L.S. Young

Posterabstract:

Epstein-Barr virus (EBV) contributes to the pathogenesis of several tumours, notably 40% of classic Hodgkin's lymphoma cases and 100% of WHO type III undifferentiated nasopharyngeal carcinomas (NPC), wherein EBV adopts a latent infection. The viral latent membrane proteins LMP1, LMP2A and LMP2B are expressed in both type II and type III latent infections with variable expression of LMP1 in NPC. A growing body of evidence indicates that EBV latent proteins can interact with cellular proteins and play important roles in tumour cell proliferation, invasion and migration. Moreover, recent studies demonstrated that EBV latent membrane proteins can interact with each other, however the molecular mechanisms underlying these interactions are still unclear. In order to elucidate further the mechanisms and biological consequences of these complex interactions in the context of a whole EBV infection, serial EBV genome delta LMP1, delta LMP2A, delta LMP2B, delta (LMP1/LMP2A), delta (LMP2A/LMP2B) and delta (LMP1/LMP2A/LMP2B) knock-out mutants have been generated by homologous recombination technology in vitro (GET method). These mutants have been used to stably transduce the epithelial cell lines AdAH, CNE (EBV-) and HONE1 (EBV-). Cell morphology and migration behaviour studies and micro-array profiling are currently under way in order to elucidate further the role of these proteins in tumourigenesis.

80 (RegID: 1515; 1516)

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EPSTEIN-BARR VIRUS DNASE INDUCES MICRONUCLEUS FORMATION, DNA STRAND BREAKS AND GENETIC MUTATION IN HUMAN EPITHELIAL CELLS

Chung-Chun Wu, Ming-Tsan Liu, Yu-Ting Chang, Chih-Yeu Fang, Sheng-Ping Chou,Hsin-Wei Liao, Kuan-Lin Kuo, Shih-Lung Hsu, Yi-Ren Chen, Shu-Feng Lin, Ching-Hwa Tsai, Yao Chang, Kenzo Takada, Jen-Yang Chen

Posterabstract:

Serological studies indicate that EBV reactivation may be associated with the development of nasopharyngeal carcinoma (NPC). Previous investigations indicated that latent EBV infection may induce genomic instability and contribute to the development of B cell tumor formation. In this study, we sought to investigate whether the lytic proteins of EBV could cause the genomic instability in human epithelial cells. In EBV-harboring NPC cells, induction of EBV lytic replication by 12-o-tetradecanoyl-phorbol-1,3-acetate and sodium butyrate or the EBV immediate early protein Zta induced DNA stand breaks and micronucleus formation. Transfection of a plasmid expressing Zta siRNA prior to induction of EBV replication prevented these changes. Furthermore, examination of a panel of EBV lytic proteins identified EBV DNase as a potent activator which induced DNA strand breaks and micronucleus formation in a dose-dependent manner. However, the expression of a DNase-null plasmid did not show similar effects. In addition, this enzyme also increased the microsatellite instability and frequency of genetic mutation in human epithelial cells. We propose that reactivation of EBV can induce genomic instability through lytic proteins such as EBV DNase, causing DNA damage, micronucleus formation, microsatellite instability and genetic mutation, and may contribute subsequently to the tumorigenesis of human epithelial cells.

81 (RegID: 1524)
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THE BPLF1, LARGE TEGUMENT PROTEIN OF EPSTEIN-BARR VIRUS, IS A POTENT UBIQUITIN SPECIFIC PROTEASE AND DENEDDYLASE.

Stefano Gastaldello, Sebastian Hildebrand, Ramakrishna Sompallae and Maria G. Masucci.

Posterabstract:

Modification by covalent conjugation of ubiquitin (Ub) or ubiquitin-like proteins (UbLs such as SUMO, Nedd8, ISG15) regulates the function, subcellular localization and turnover of cellular proteins. The activity of these protein-modification systems is regulated by substrate-specific enzymes that mediate the conjugation (E3 enzymes) or deconjugation (Ub and UbL-specific proteases) of the modifiers. These enzyme families are often targeted by viruses that exploit their activity to remodel the cellular environment in favour of their own persistence and replication. Recent evidence suggests that the BPLF1 open reading frame of the Epstein-Barr virus genome encodes a ubiquitin-specific protease. BPLF1 is a tegument protein that is expressed during the late phase of the virus replicative cycle. The role of its deconjugases activity in the context of virus replication and assembly or as a modifier of cellular functions is unknown. We have studied the enzymatic activity of BPLF1 against a variety of reporter substrates and in vivo disassembled Ub and UbL conjugates. We have fund that the N-terminal portion of BPLF1 (BPLF1-N) is a very potent deconjugase that cleaves Ub-AMC with a KM 10 fold higher compared to a panel of human enzymes. In addition, BPLF1-N was equally active against Neddylated substrates including Nedd8-AMC and Nedd8-GFP as well as in vivo Neddylated proteins.

82 (RegID: 1674)
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HISTONE MODIFICATION ON EBV LYTIC REPLICATION ORIGIN (ORILYT) UPON REACTIVATION

Yi-Hsuan Tsi, Chia-Chi Ko, Ting-Yu Chang, Ren-Hao Chen, and Chi-Ju Chen

Posterabstract:
EBV genome is maintained as a chromosome-like episome at latency. It is thought that lytic replication initiates with the theta (θ)-form replication and switches to roll-circle mode later in viral DNA replication. However, the cellular and viral activities involved in the transition from histone-associated genome to θ-replication remain unclear. Since it is spaciously impossible for viral replication machinery binding on a compact oriLyt, we hypothesized that cellular activities changing DNA-histone position at EBV OriLyt are involved in initiating the viral DNA replication upon reactivation.
Histone H3 acetylation of lysine 9 and 14 is implicated in transcription activation, while tri-methylation of histone H3 lysine 9 is associated with transcription repression. Here we showed that the level of acetylation of histone H3 lysine 9 and 14 on oriLyt elevated significantly upon viral reactivation in Raji cells. On the contrary, tri-methylation of histone H3 lysine 9 was reduced upon viral reactivation. The reversal of the repressive histone H3-lysine 9- methylation was accompanied with the increase of the acetylation on the same residue. Surprisingly phosphorylation of H3 serine 10, which only affects transcription activation of a subset of genes during interphase, also increased on OriLyt upon viral activation. The possible involvement of cellular histone modification enzymes, such as CBP and MSK1, were also studied. The results suggest that EBV adapted cellular histone modification machinery to initiate its DNA replication on OriLyt.

83 (RegID: 1708; 1710; 1711)

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OCT2 AND EBNA 1 IN THE CONTROL OF SWITCHING BETWEEN LATENCY PROGRAMS

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1) Theoretical Biological Physics, Dept of Physics, Royal Shool of Technology, Stockholm,Sweden; 2) Department of Clinical Chemistry and Transfusion Medicine Sahlgrenska University Hospital Goteburg Sweden;

Posterabstract:

The switch between latancy programs I/II and III EBV have major implications for cell cycle entry and exit, and thus for viral pathogenesis. The family of repeats (FR) is the major upstream control element of the EBV latent C promoter (Cp), driving latency III and cell proliferation. It can be activated by the binding of multiple EBV nuclear protein EBNA1s. We have earlier shown that OCT-transcription factors also can bind to and activate FR, as demonstrated by electrophoretic mobility shift assay (EMSA), by an affinity DNA-binding assay ("DNA-fishing"), chromatine immuno precipitation (ChIP) and luciferase based reporter assays. We have also shown that OCT proteins turned into repressors by Grg/TLE can repress promoter activity through EBVs FR. We have knocked down Oct2 levels in latency I cells, which affects EBNA1 expression levels. A mechanical statistical model has been developed for this switch and its control by EBNA1 and Oct2. From the model can be inferred the key regulatory events and numbers of molecules required for the switch to occur. With this model we propose one mechanism for switching between EBV driven cell cycle entry or exit, as consequencies of the latency program switches.

84 (RegID: 1744)
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A MUTANT EBV CONTAINING A STOP CODON INSERTED INTO THE VIRAL PK OPEN READING FRAME CAN REPLICATE INTRACELLULARLY, BUT IS HIGHLY IMPAIRED FOR RELEASE OF INFECTIOUS VIRAL PARTICLES

Qiao Meng, Stacy Hagemeier, Shannon C. Kenney

Posterabstract:
The EBV BGLF4 gene encodes a serine/threonine protein kinase that is homologous to the CMV UL97 kinase. Although EBV-PK phosphorylates a number of different viral and cellular proteins in vitro (including BMRF1), its role(s) in the context of the intact viral genome has not been well studied. We created a PK-mutant virus by inserting stop codons at residues 1 and 5 in the EBV-PK open reading in the EBV bacterial artificial chromosome. The phenotype of the wild-type (wt) virus versus the EBV-PK stop mutant was examined in 293 cells, as well as 293T cells. Cells containing the PK-stop mutant did not have the hyperphosphorylated form of the BMRF1 protein, confirming loss of EBV-PK function. By southern blot analysis, the PK-stop mutant virus replicated at least as well as the wt virus in 293 cells. However, the amount of infectious virus released into the supernatant of BZLF1-transfected 293 cells (detected by the green Raji unit assay) was dramatically reduced in the cells containing the PK-stop mutant. This phenotype was reversed when the PK protein was expressed in trans. The expression level of a variety of different IE (BZLF1 and BRLF1), early (BMRF1, SM, TK, BFRF1 and BFLF2) and late (BcLF1) viral proteins was not significantly different in 293 cells infected with wt versus PK-stop mutant viruses. Surprisingly, in 293T cells, we found that cells infected with the PK-stop virus produced almost as much infectious virus as cells infected with the wt virus. Furthermore, transfection with a T antigen expression vector partially rescued the ability of 293 cells containing the PK-stop mutant to release infectious viral particles. These results suggest that EBV-PK is not essential for replication in 293 cells, but is impaired for the ability to release infectious viral particles, and this defect can be rescued by the SV40 T antigen.

85 (RegID: 1764; 1765; 1766; 1767)

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CELLULAR PROTEINS ZEB1 AND ZEB2/SIP1 PLAY MAJOR ROLES IN REGULATING THE LATENT-LYTIC SWITCH BZLF1 GENE OF EBV

Amy L. Ellis, Xianming Yu, Zhenxun Wang, Shannon C. Kenney, and Janet E. Mertz

Posterabstract:

The EBV-encoded protein Zta plays a central role in regulating the switch between latency and lytic replication. We previously identified a silencing element in the BZLF1 promoter (Zp) which we named ZV. We showed that a cellular factor, ZEB1, could bind to the ZV element, repressing BZLF1 gene expression (Kraus et al., J. Virol. 77:199, 2003). Furthermore, mutation of the ZV element in the context of a whole genome of EBV strain B95.8 led to spontaneous reactivation out of latency in epithelial 293 cells, with production of infectious virus (Yu et al, PLoS Pathogens 3(12) e194, 2007).

We now show that EBV-infected 293 cells express not only ZEB1, but also ZEB2/SIP1, another zinc-finger E-box binding protein that can bind the Zp ZV element, repressing BZLF1 gene expression. While addition of either ZEB1 + ZEB2 siRNAs or the activators TPA + butyrate failed to lead to reactivation out of latency of wild-type EBV-infected 293 cells, addition of ZEB2 siRNAs and TPA+butyrate together did. Thus, several factors need to change to activate Zp expression.

Analysis of multiple EBV-positive epithelial and B-lymphocytic cell lines indicated that ZEB1 was more ubiquitously expressed than ZEB2. In ZEB1-positive, ZEB2-negative cell lines, the addition of ZEB1 siRNAs alone were sufficient to activate lytic EBV protein expression. On the other hand, in most ZEB1- and ZEB2-positive cell lines, ZEB1 siRNAs played only a minor role in reactivation; only ZEB2 siRNA + TPA+butyrate lead to lytic protein expression. In gastric AGS cells that lack both ZEB1 and ZEB2, infection by EBV leads to chronic lytic infection (Feng et al., J. Virol. 81:10113, 2007). Thus, we conclude that both ZEB1 and ZEB2 play central roles in maintenance of EBV latency. However, other factors such as MEF2D and the ZIIR-binding protein contribute as well to silencing BZLF1 gene expression.

86 (RegID: 1921; 1922)

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CHARACTERIZATION OF LMP-1 BEHAVIOR IN LIVE CELLS

Ryan Takeshita, Chris Stockburger, and Jennifer Martin (Univ. of Colorado at Boulder)

Posterabstract:

Latent Membrane Protein-1, the EBV oncoprotein essential for B cell immortalization, constitutively mimics the signaling activity of the ligand-dependent CD40 receptor. Upon binding to its ligand, CD40 homo-trimerizes and migrates to lipid rafts (membrane microdomains rich in signaling molecules) in order to generate a functional signaling complex. Because LMP-1 and CD40 trigger similar signaling pathways (NFκB, JAK/STAT, JNK, etc.), and based on evidence from biochemical experiments (ie: co-immunoprecipitation and ultracentrifugation of cellular lystates in sucrose gradients), most of the assumptions regarding LMP-1 complex formation are rooted in analogy to that of CD40. However, there is little known about the behavior of LMP-1 in live, intact cells.

In an effort to characterize the behaviors of LMP-1 necessary for LMP-1 signaling and, in turn, EBV-driven B cell immortalization, we utilize a variety of high-resolution confocal fluorescence microscopy techniques in live cells (epithelial and lymphoid). We show that LMP-1 homo-oligomerizes by Förster Resonance Energy Transfer and describe the stoichiometry and subcellular localization of these LMP-1 clusters. We also investigate the relationship between oligomerization, lipid raft-association, and LMP-1 signaling by measuring the effect of cholesterol depletion upon LMP-1 homo-oligomerization and LMP-1's ability to induce NFκB. By using live-cell imaging techniques, we can move away from studying LMP-1 based on its mimicry of activated CD40 and towards the aspects of LMP-1 that make it unique from CD40. This transition will assist in the creation of therapies for EBV-related malignancies without side-effects on endogenous CD40 activity.

87 (RegID: 1989)
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for poster presentation in “Proteomics and viral replication” session:

CELLULAR FACTOR NF-Y PLAYS AN IMPORTANT ROLE IN LYTIC GENOME REPLICATION OF MURINE GAMMAHERPESVIRUS-68

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Posterabstract：
Murine gammaherpesvirus 68 (MHV-68) replicates robustly in cell culture, providing a model for studying viral genome replication during de novo infection of tumor-associated herpesviruses. Employing a de novo infection-replication assay, we previously identified a 1.25-kb fragment towards the right end of MHV-68 genome that contains an origin of lytic replication. We report here our identification and characterization of a second MHV-68 oriLyt. We first fine-mapped cis-elements from the right oriLyt that are essential for mediating DNA replication, using a transposon-mediated high-density mutagenesis method. A small fragment located towards the left end of MHV-68 genome was found to contain these essential elements. We cloned a 1.2-kb sequence containing this small fragment and showed through the de novo infection-replication assay that it indeed functions as an oriLyt. We further analyzed this left oriLyt by scanning deletion analysis and site-directed mutations. We found that CCAAT motifs are essential for oriLyt function and that an AT-rich region also plays a role in lytic replication. However, GC-rich repeats are not key cis-element of MHV-68 oriLyt. Moreover, we showed that, NF-Y, a ubiquitous trimeric cellular transcription factor, binds to CCAAT boxes in EMSA and ChIP assay. Using a dominant negative expression plasmid, we demonstrated that NF-Y plays an important role in mediating MHV-68 genome replication during de novo infection. To our knowledge, this is the first time that NF-Y is implicated in DNA replication. Our findings broaden the understanding of NF-Y functions and may provide new insights into mechanism of herpesvirus lytic DNA replication.

88 (RegID: 1167)
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INTERACTION BETWEEN THE NACOS CELLULAR PROTEIN UBINUCLEIN AND THE EPSTEIN-BARR-VIRUS TRANSCRIPTION FACTOR EB1 IN EBV-INFECTED EPITHELIAL CELLS

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Posterabstract:
EBV replication is preferentially observed in terminally differentiated lymphoid or epithelial cells. It is now well known that the switch from latency to productive cycle of EBV is dependent on the expression of a viral transcription factors called EB1 (ZEBRA or Zta). We have previously identified a cellular protein of unknown function, called Ubinuclein (Ubn), whose interaction with the EB1 basic domain inhibits the formation of EB1-DNA complexes in vitro.
Here, we found that Ubn is nuclear in 293HEK, HeLa, non-confluent MDCK or in keratinocyte cells in culture, but is found exclusively in the tight junctions of confluent MDCK or in polarized HT29 epithelial cells where it colocalized with the tight junction markers ZO1, Claudin1 or Occludin. In accordance with this, we have shown by GST-pull down experiments and co-immunoprecipitation in vivo, that Ubn directly interacts with the members of the MAGUK (Membrane Associated Guanylate Kinase) family, ZO1, ZO2, and ZO3, which are part of the tight junction molecular complex. Thus, Ubn has a dual localization in epithelial cells: nuclear in undifferentiated or non-confluent cells, but relocalized to the tight junctions when cells reach confluency. Hence, Ubn is a new member of the NaCos protein family (Nuclear and adhesion complex components).
Furthermore, we show that in the epithelial HEK293 cells infected by EBV and induced to the productive cycle, Ubn over-expression decreased viral DNA replication and viral particles production. In these conditions, expression of the EB1 early gene targets was also decreased. In addition, when Ubn expression was inhibited by siRNA, the amount of EBV particles produced was increased. Moreover, in latently EBV-infected undifferentiated oro-pharyngeal C666-1 or gastric AGS epithelial cells, Ubn is exclusively expressed in tight junctions. These data suggest that in vivo, Ubn could interfere with EB1's activity.
Taken together, these results suggest a possible role of Ubn in EBV's reactivation in epithelial cells, which may be modulated by the subcellular localization of Ubn.

89 (RegID: 1184; 1185; 1186)

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FUNCTIONAL INTERPLAY BETWEEN EBV REPLICATION PROTEIN AND THE HOST DNA-DAMAGE RESPONSE PROTEIN 53BP1

Sarah G Bailey, Elizabeth Verrall, Questa H Karlsson, Celine Schelcher, Aiden Doherty and Alison J Sinclair.

Posterabstract:

EBV is encoded by a double-strand DNA genome that undergoes lytic replication in the nucleus. Although the virus encodes a DNA polymerase and accessory proteins, there is the potential requirement for further contributions from the host cell replication machinery. Indeed, EBV activates ATM signal transduction in a marmoset cell line and murine gamma-herpesvirus (MHV68) requires ATM and a component of its signal transduction pathway, H2AX, for its infection cycle. ATM is part of the DNA-damage response pathway involved in transmitting signals from DNA with atypical structure.

Here we investigate the role of the ATM signal transduction pathway for EBV lytic replication. Using a specific pharmacological inhibitor, KU-55933, we establish a role for ATM in EBV genome replication in human lymphoblastoid cell lines. A global proteomics approach identified a point of direct interaction between EBV and the ATM signal transduction pathway through the physical interaction of Zta (BZLF1, ZEBRA, Z) with 53BP1. 53BP1 is a large protein, with multiple binding partners, which may alter the function or location of its interacting proteins.

The switch between viral latency and the lytic cycle is governed by a multifunctional viral protein Zta (BZLF1, ZEBRA); in part through its ability to bind to the EBV origin of lytic replication.

We demonstrate the importance of the 53BP1/Zta interaction for viral replication by:
(i) The reduction in viral lytic replication observed following SiRNA knock-down of 53BP1 expression;
(ii) Generating mutants of Zta that are deficient for interaction with 53BP1 and replication but otherwise competent.

Possible roles for the 53BP1/Zta interaction include opening chromatin structure at the origin of lytic replication or modulating the cell-cycle arrest or pro-apoptotic arms of ATM signal transduction. The identification of a requirement for ATM signal transduction for EBV lytic replication provides potential strategies to specifically modulate viral replication in vivo.

90 (RegID: 1207)
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BDLF2 ENCODES THE ELEVENTH EBV VIRION ENVELOPE GLYCOPROTEIN AND IS DEPENDENT ON BMRF2 FOR PROCESSING AND TRANSPORT

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Posterabstract:
Ten glycoproteins are currently said to be present in the envelope of EBV. However, in silico analysis suggests that there may in fact be eleven. The BDLF2 gene, which proteomic analysis has identified as a tegument protein, is instead predicted to encode a type II membrane protein with six potential N-linked glycosylation sites in its carboxyterminal ectodomain. In the related murine gammaherpesvirus 68, the homolog of BDLF2, ORF27 or gp48, interacts with a second glycoprotein, ORF58, a homolog of EBV BMRF2. To characterize the BDLF2 gene product further, antibody was made to GST-fusions of both the aminoterminal and carboxyterminal domains of BDLF2 and the loop of BMRF2 which contains an RGD motif. BDLF2 and BMRF2 were also cloned for expression in mammalian cells. Indirect immunofluorescence staining demonstrated a globular accumulation of protein in cells expressing BDLF2 alone, but in cells expressing both BDLF2 and BMRF2 the BDLF2 protein relocalized to a smooth rim at the plasma membrane. Western blotting revealed that when expressed alone BDLF2 is a protein of 68 kDa and can be digested with endoglycosidase H to 46 kDa, the predicted size of the protein backbone. However, when expressed together with BMRF2, BDLF2 carries endoglycosidase H resistant sugars. In addition to full-length BDLF2, both amino- and carboxyterminal fragments are found in purified EBV virions. These are of a size consistent with cleavage at a potential furin or related proprotein convertase site. Antibody to BMRF2 is able to immunoprecipitate full length BDLF2 and the aminoterminal, but not the carboxyterminal cleavage product. Together these data suggest that BDLF2 is an eleventh glycosylated virion envelope protein that complexes with and is dependent on BMRF2 for processing and transport. The carboxyterminal cleavage product of BDLF2, however, may interact with additional viral or cellular proteins.

91 (RegID: 1261)
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EPSTEIN-BARR VIRUS SM PROTEIN INHIBITS BHRF1 (VIRAL BCL-2) EXPRESSION BY DOWNREGULATING THE EBV TRANSCRIPTIONAL ACTIVATOR R PROTEIN.

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Posterabstract:

During primary infection and during reactivation from latent infection, EBV undergoes productive lytic replication. The switch from latency to lytic replication is regulated by two EBV transcriptional activators; BZLF1 (Z, Zta, EB1) and BRLF1(R, Rta). SM is an RNA-binding EBV protein expressed after Z and R during the lytic cycle and is essential for virus production. SM enhances EBV mRNA accumulation via multiple mechanisms that enhance target mRNA stability, processing and export. We have previously compared EBV gene expression in the presence and absence of SM using an epithelial cell line carrying an EBV recombinant deleted for SM. We found that most EBV genes are upregulated by SM, but one gene, BHRF1, is highly downregulated. BHRF1 is an anti-apoptotic protein that is homologous to cellular Bcl-2 and is expressed shortly after lytic EBV replication begins.

To ask whether the inhibitory effect of SM on BHRF1 expression was post-transcriptional, we measured expression of BHRF1 from a heterologous promoter. SM was unable to decrease BHRF1 gene expression from a CMV promoter, suggesting that the inhibitory effect was promoter-dependent. We therefore analysed BHRF1 promoter activity with luciferase reporter assays and found that SM downregulated BHRF1 promoter activity, but only in EBV-positive cells, suggesting involvement of other EBV proteins in BHRF1 promoter inhibition by SM. We further demonstrate that SM downregulates R protein and RNA levels in EBV positive cells and that the majority of BHRF1 promoter activity in 293 cells is R-dependent. Our results demonstrate that SM may inhibit BHRF1 promoter activity by downregulating R expression during the lytic phase of EBV replication and may play a role in feedback modulation of lytic cycle gene expression. Temporal modulation of BHRF1 levels during lytic replication may thus be important for maintaining cell viability for a limited period during the early stage of EBV replication.

92 (RegID: 1280)
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EPSTEIN-BARR VIRUS REPLICATION IS REGULATED BY THE VIRAL PROTEIN LF2

Michael A. Calderwood, Amy M. Holthaus, Andreas Heilmann and Eric Johannsen

Posterabstract:
The switch from latent to lytic EBV replication is regulated by two immediate early genes BZLF1 and BRLF1, which encode the transcriptional activators Z (Zta) and R (Rta). Both Zta and Rta are essential for EBV replication and viral genomes deleted for either BZLF1 or BRLF1 are not competent for viral DNA replication or virion production. We previously determined that the LF2 gene encodes an Rta binding protein. We now report that LF2 can block Rta activation of multiple lytic promoters in both epithelial and B cells. However some promoters, notably the Rta promoter, are resistant to LF2. Furthermore, in EBV infected epithelial cells, neither Zta nor Rta can induce replication in the presence of LF2. We also find that LF2 specifically induces modification of Rta by the small ubiquitin-like modifiers, SUMO2 and SUMO3. LF2 decreases Rta activation by at least two means: decreased DNA binding and interference with transcriptional activation by the Rta acidic activation domain. Our results demonstrate that LF2, a gene deleted from the B95-8 EBV reference strain, encodes a potent inhibitor of EBV replication and suggest that future studies of EBV replication need to account for the potential effects of LF2 on Rta activity.

93 (RegID:1449; 1450; 1451)

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LMP1 IS NOT REQUIRED FOR LYTIC EBV REPLICATION IN EPITHELIAL CELLS

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Posterabstract:

Latent Membrane Protein-1 (LMP1) is a viral oncogene expressed in most Epstein-Barr Virus (EBV)-related malignancies. Relatively little is known about the role of LMP1 in epithelial cells and conflicting evidence exists for the role of LMP1 in the lytic cycle. Here, we report that LMP1-deleted virus replicates as efficiently as wt in epithelial cells. In this study, we engineered a Bacterial Artificial Chromosome (BAC) which produces recombinant viruses that do not encode LMP1. After introduction of the EBV-BAC into HEK293 epithelial cells, cell lines are selected for the stable maintenance of an episomal EBV genome. Following lytic cycle induction with BZLF1, we detect no impairments in EBV lytic protein expression, replication, encapsidation, and egress. Trans-complementation with a plasmid encoding the ORF of LMP1 does not increase viral protein expression or replication. As anticipated, virus from the cell-free supernatant from wt but not LMP1-deleted cells is capable of transforming PBMCs. These data demonstrate that LMP1 is not required for viral replication in epithelial cells.

94 (RegID: 1743)

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BZLF1 (Z) ONLY BINDS TO, AND ACTIVATES, THE METHYLATED FORM OF THE EBV BRRF1 (NA) PROMOTER

Sarah J. Dickerson, Amanda R. Robinson, William T. Seaman, Shannon C. Kenney

Posterabstract:

BZLF1 (Z), a bZip protein, binds to the AP1 motif, as well as atypical AP1-like motifs known as Z-responsive elements (ZREs), and induces lytic viral reactivation. A unique feature of Z is its unusual ability to preferentially bind to, and activate, the methylated form of the immediate-early (IE) BRLF1 promoter (Rp), which contains atypical CpG-containing ZREs. A Z mutant, Z(S186A), altered at serine 186 in the DNA binding domain cannot bind to methylated ZREs in Rp but binds to AP1 motifs. To date, only two ZREs in the entire EBV genome (both within Rp) have been shown to contain CpG motifs. The BRRF1 gene product, Na (encoded within the opposite strand of the BRLF1 intron), enhances BRLF1-mediated viral reactivation. Here we demonstrate that the BRRF1 promoter (Nap) has two CpG-containing ZREs that are only bound by Z in the methylated form. Only the methylated form of Nap can be activated by Z in reporter gene assays, and this activation requires both of the methylated ZREs in Nap. The requirement of promoter methylation for Z activation is much more profound for Nap than Rp, likely reflecting that Z binds to both the unmethylated and methylated forms of Rp ZREs. Z(S186A) cannot bind to the methylated Nap ZREs, and cannot activate Nap in reporter gene assays. These results are the first to identify functionally important ZREs in the EBV genome that can only be bound by Z in the methylated form. The apparently unique ability of Z to activate the methylated forms of the R and Na promoters, but not other lytic EBV promoters (in which the known ZREs do not contain CpG motifs), may be a mechanism by which the virus ensures that R and Na are the first genes activated during Z-mediated reactivation in cells with a methylated viral genome.

95 (RegID: 1752; 1754; 1755; 1756; 1757)

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STRONGER BINDING OF EPSTEIN-BARR VIRUS ZEBRA PROTEIN TO DNA IS REQUIRED FOR INITIATION OF VIRAL REPLICATION THAN FOR ACTIVATION OF TRANSCRIPTION

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Posterabstract:

ZEBRA is an essential component of the EBV lytic replication machinery. It interacts with the origin of lytic replication (oriLyt) and forms multiple protein-protein interactions that lead to the assembly of replication complexes on oriLyt. We previously showed that phosphorylation of ZEBRA at S173, a residue upstream of the DNA binding domain, was essential for viral replication but not transcriptional activation of early lytic genes, such as the BRLF1 gene which encodes the Rta protein. The importance of phosphorylation of S173 in viral replication was attributed to its role in modulating the DNA binding activity of ZEBRA. Lack of phosphorylation at this residue reduced the DNA binding activity of ZEBRA in vitro and its capacity to associate with oriLyt and with the Rta promoter (Rp) in vivo. This finding suggested that activation of transcription tolerates weak association while initiation of replication requires stronger association of ZEBRA with DNA. To investigate the proposed link between DNA binding and initiation of lytic DNA replication we screened a library of 54 ZEBRA mutants, with single point mutations in the DNA recognition domain, for proteins that were competent in transcription but defective in replication. Three ZEBRA mutants were identified. We then asked whether these mutants share the same defect as Z(S173A) in binding DNA in vitro and in vivo. We found that all replication defective mutants shared the same weak affinity to oriLyt and Rp in vivo. However, over-expression of the six EBV replication proteins augmented association of ZEBRA with oriLyt. This augmentation partially suppressed the replication defect of these mutants. Our findings indicate that strong association between ZEBRA and oriLyt is mandatory for initiation of viral replication. Moreover, one or more component of the six EBV replication proteins plays a role in stabilizing association of ZEBRA with oriLyt.

96 (RegID: 1753)

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EXPRESSION OF THE EBV BRRF1 GENE PRODUCT, NA, IS SUFFICIENT TO INDUCE THE LATENT TO LYTIC SWITCH IN SOME EPITHELIAL CELL LINES.

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Posterabstract:

High level expression of either EBV immediate-early protein, BZLF1 (Z) and BRLF1 (R), is sufficient to induce the switch from the latent to lytic form of EBV infection in many EBV-infected cell lines. Z and R transcriptionally activate one another's promoters, and together can activate the entire EBV lytic cascade. However, efficient R activation of lytic EBV gene expression in latently infected 293 cells (but not B cells) was previously shown to require the BRRF1 gene product, Na (Hong et al 2004), which is encoded on the opposite strand of the BRLF1 intron. Furthermore, Na alone activates the BZLF1 promoter (Zp) in reporter gene assays through its effect on the Zp ZII motif (a c-jun binding site), and induces c-jun phosphorylation. In this study, we have investigated whether high level Na expression is sufficient to induce the latent to lytic switch in certain EBV-infected cell lines. We show that Na expression is sufficient to induce lytic viral protein expression in both Hone-Akata cells (an NPC line) and AGS-Akata cells (a gastric carcinoma line). Since Na interacts with TRAF2 in a yeast two-hybrid analysis (PNAS 104:7606-7611), and TRAF2-dependent signaling can activate c-jun phoshorylation, we investigated whether TRAF2 and Na interact within human cells. We found that over-expressed TRAF2 and Na can be co-immunoprecipitated, suggesting that these two proteins do interact in vivo. Furthermore, when endogenous TRAF2 expression was decreased using siRNA, the ability of Na to cooperate with R to induce lytic infection in EBV-293 cells was much decreased. Finally, preliminary results indicate that the mRNA for Na is transcribed with immediate-early kinetics during primary infection. These results suggest the BRRF1 gene product, Na, plays a much more important role than previously recognized in promoting lytic gene expression in epithelial cells.

97 (RegID: 1753)
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SUMOYLATION OF Z LYSINE 12 INHIBITS Z TRANSCRIPTIONAL FUNCTION.

Stacy R. Hagemeier, Sarah J. Dickerson, Qiao Meng, Xianming Yu, Janet E. Mertz, Shannon C. Kenney

Posterabstract:
The immediate-early protein BZLF1 (Z) is a transcriptional activator of EBV early genes and induces the switch from the latent to lytic form of EBV infection. In addition to its transcriptional effects, Z binding to the lytic origin of replication is required for lytic viral replication. Our lab has previously reported that Z is post-translationally modified with small ubiquitin-related modifier-1 (SUMO-1) at lysine 12. Sumoylation of cellular proteins has been shown to have a variety of different effects, including alteration of cellular localization, stability and transcriptional activity. However, the effect of Z sumoylation on Z functions during EBV infection currently remains unclear, since the previous Z mutant used to study Z sumoylation is altered at both residues 12 and 13. To further examine the role of Z sumoylation, we constructed Z mutants (in the SG5-Z expression vector) which switched lysine 12 to either an alanine or arginine. Both of these sumo-defective Z mutants induced more early lytic gene expression than wildtype Z when transfected into 293 cells stably infected with a Z-KO virus, and produced at least as much infectious virus. Furthermore, we showed that Z is not only sumoylated by SUMO-1, but also by SUMO-3. To examine how sumoylation effects Z function in the context of the intact virus, we have generated clones of 293 cells containing recombinant EBV genomes in which the Z lysine at amino acid 12 has been mutated to either an alanine (ZK12A) or arginine (ZK12R). Our preliminary studies indicate that loss of Z sumoylation enhances the ability of the virus to be induced for lytic replication when the mutant cell lines are treated with the phorbol ester 12-tetradecanoyl-phorbol-13-acetate (TPA) and sodium butyrate. These results suggest that sumoylation of Z helps to promote viral latency by inhibiting Z transcriptional function.

98 (RegID: 1758)

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THE B-CELL SPECIFIC TRANSCRIPTION FACTOR, OCT2, INHIBITS LYTIC EBV REACTIVATION.

Amanda R. Robinson, Shannon C. Kenney

Posterabstract:

The EBV latent to lytic switch is mediated by the BZLF1 (Z) and BRLF1 (R) immediate-early (IE) viral proteins, driven by the BZLF1 (Zp) and BRLF1 (Rp) IE promoters, respectively. Studies in humans suggest that EBV is normally latent in B-cells, but reactivates when B-cells differentiate into plasma cells. Recently the cellular transcription factor XBP1, which is activated by plasma cell differentiation, was shown to induce EBV reactivation through activation of Zp and Rp. However, other than ZEB1, cellular factors which promote viral latency in B-cells are not well studied. Here we demonstrate that the B-cell specific transcription factor, Oct2, negatively regulates EBV reactivation. In co-transfection assays, Oct2, but not Oct1, inhibited Z-mediated lytic viral reactivation in Hone-Akata cells, while not decreasing the level of transfected Z protein. Oct2 also inhibited Z-mediated activation of Zp- and Rp- driven reporter gene constructs, although it did not affect the level of constitutive Zp or Rp activity. We found that both Zp and Rp contain Oct2 binding sites, and that Oct2 binding sites in Zp overlap with known Z-binding motifs (ZREs). Finally, we have also shown that the Oct2 protein can be co-immunoprecipitated with the Z protein when both are over-expressed in HeLa cells. These results are the first to identify a potential role for Oct2 as a negative regulator of lytic EBV reactivation. Direct interactions between BZLF1 and the B-cell specific transcription factor, Oct2, may help EBV to establish latency in a B-cell specific manner.

NOTES:

Poster Sessions

Session 3：Genetics, Non-coding RNAs and epigenetic

99 (RegID: 1135; 1136)

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POSSIBLE ROLE FOR EBERS IN THE REGULATION OF CCR7 EXPRESSION IN EBV-INFECTED B CELLS

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Posterabstract:

The Epstein-Barr virus-encoded proteins EBNA-2 and LMP1 have been shown to modulate the expression of chemokine receptors CXCR4 and CCR7. These chemokine receptors play an essential role in B cell trafficking and micro-anatomic organization of lymphoid tissues. Previously, it was shown that introduction of EBNA-2 into the EBV-negative Burkitt lymphoma (BL) line BL41 resulted in the up-regulation of CCR7. In the ER/EB2-5 system, where the EBNA-2 expression and activity is estrogen dependent, CCR7 was down-regulated after 3 days of estrogen starvation. These data indicate that EBNA-2 may play a role in the regulation of CCR7 expression.

We have investigated the CCR7 expression in tonsillar B cells after EBV infection. One week after infection CCR7 expression was markedly decreased even though EBNA-2 was expressed. This paradoxical result may be due to the involvement of other EBV-encoded proteins or miRNAs in the primary infected B cells.

In the present study we have examined the effect of the EBV-encoded small RNAs, EBERs, on the expression of CCR7 in the EBV-negative Akata BL transfected with an EBERs expression vector. CCR7 expression decreased in the EBERs-expressing BL cells in comparison with its control. This finding is consistent with the possibility that EBERs may counteract the EBNA-2-mediated CCR7 induction in the primary EBV-infected tonsillar B cells.

100 (RegID: 1164)
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RECOMBINANT EBVS REVEAL COOPERATIVE REGULATION OF CELLULAR GENES BY THE EBNA3 PROTEINS.

Rob E White, Jade Yee, Emma Anderton, Martin J Allday

Posterabstract:
EBNAs 3A, 3B and 3C all bind transcriptional repressors and have been implicated in the regulation of EBV and host promoters. Using a B95-8 BAC system, we have generated recombinant viruses (and their revertants) in which single EBNA3 genes are deleted, and another in which the whole EBNA3 locus is deleted (E3KO). BL31 cells were infected with these recombinants to generate cell lines carrying parental, mutant or revertant EBV. Western blot analysis of EBV latent proteins suggests that there is no consistent modulation of EBV genes in the single knock-outs, but E3KO lines have reduced LMP1 expression.
The cell lines were subjected to microarray analysis using Affymetrix Exon chips. Of the ~22,000 genes in the core set, around 1000 are differentially expressed >2-fold (similar numbers induced and repressed) in cells carrying a single gene mutant as compared to the parental and revertant viruses. Most of these genes are also differentially regulated by E3KO. The contribution of EBNA3C >EBNA3B >EBNA3A, but most strikingly there appears to be an enormous amount of cooperation between the EBNA3s, with around half of the differentially expressed genes dependent on more than one EBNA3 protein. Gene groups regulated include those involved with B-cell differentiation, Ras-signaling, interferon responses and apoptosis (including Bim/BCL2L11). Some genes previously characterized as EBNA2 targets (eg CD23), also appear to require EBNA3s for their regulation by EBV – implying wider co-operation between the EBV latent proteins. Furthermore, we generated recombinant mutant viruses whose EBNA3A and/or 3C are unable to bind to the transcriptional repressor CtBP. BL31 cells containing viruses whose EBNA3s are unable to bind CtBP differentially regulate around 50 genes (which are also regulated by gene knockout), identifying a subset of genes or gene networks that require the recruitment of CtBP by EBNA3s for their modulation by EBV.

101 (RegID: 1265; 1289)

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GENOME WIDE TRANSCRIPTIONAL AND METHYLATION PROFILING REVEALS THAT EBV INDUCES HYPOMETHYLATION OF CELLULAR GENES IN PRIMARY B CELLS

Sarah Leonard, Wenbin Wei, Paul Murray, Ciaran Woodman

Posterabstract:

Although it is widely known that EBV can regulate its own life-cycle using the cell's epigenetic machinery, the impact of virus-induced epigenetic changes, such as DNA methylation on the expression of cellular genes has received less attention.

Genome wide expression and promoter methylation arrays were used to reveal the transcriptional and epigenetic changes which follow infection of germinal centre (GC) B cells with EBV. We have previously shown that this cell line model recapitulates many of the transcriptional changes observed in Hodgkin's lymphoma (HL).

Gene expression profiling revealed a down-regulation of the DNA methyltransferases DNMT3b and DNMT1, and up-regulation of DNMT3a in GC B cells six weeks following their infection with EBV. These transcriptional changes were associated with hypomethylation of more than 487 cellular genes. Methylation changes in genes previously implicated in carcinogenesis eg. CSMD1, SPRY2 and MAGE-A3 have since been confirmed by pyrosequencing. Given that we have also observed the same changes in transcription of these DNMTs in HL cell lines, we believe there is a compelling case for the further investigation of the contribution of these epigenetic changes to the pathogenesis of B cell neoplasia.

102 (RegID: 1281)

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TWO HIGHLY CONSERVED VIRAL GENES IN EPSTEIN - BARR VIRUS ASSOCIATED GASTRIC CARCINOMA

Wang Yun， Yang Ting-ting， Liu Xia， Luo Bing

Posterabstract:

The EBV-oncogene BARF1 (BamHI A right frame 1) was reported to have a latent expression restricted to epithelial malignancies. BHRF1 gene，a bcl-2 homologue, was found to have anti-apoptotic function. We wondered if these two genes had sequence variation involved in gastric carcinoma oncogenesis since the virus performance was utterly different between the malignant cases and healthy adults. In the present study, we looked for BARF1 and BHRF1 variants in isolates from Epstein-Barr virus associated gastric carcinoma (EBVaGC) and throat washings (TWs) of healthy adults in north of eastern China. Nest-PCR was performed for amplification of BARF1 and BHRF1 ORFs from 17 EBVaGCs and 33 EBV positive TWs，with NEC cell line as a representative isolate of nasopharyngeal carcinoma. Bidirectional solid-phase sequencing of the PCR products was employed, and sequences of respective samples were checked for homology using BLAST. Alignments between sequences were analyzed by software DNA star, using published sequences from B95-8 cell line as prototype. We found that the BARF1 gene was highly conserved in all the specimens, besides some inconsistent mutations possibly caused by mismatching in PCR amplification. A small number of missense mutations in the BHRF1 protein were found relative to the prototype B95.8 EBV sequence and these were predominantly clustered near the amino terminus of the BHRF1 protein outside conserved domains identified in the Bcl-2 homologues. In addition, NEC EBV sequence was the most divergent one to B95.8 isolate, with the homology approaching to 96.9%.Two nonsense mutation, T→C in 54615 and 54637, appeared in 14GCs /31TWs, and 14GCs /28 TWs, respectively. Neither Gly99 in BH1 nor Trp143 in BH2 was altered in all the sequences, which correspond to the functionally important Gly145 and Trp188 in Bcl-2. The highly conserved nature of BARF1 and BHRF1 amongst all specimens supports their proposed important role in the establishment of virus persistence.

103 (RegID: 1282)

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GENOTYPING OF EPSTEIN - BARR VIRUS IN EPSTEIN-BARR VIRUS ASSOCIATED GASTRIC CARCINOMA

Yang Ting-ting，Wang Yun，Liu Xia，Li Xin，Pang Zeng-chang,Luo Bing

Posterabstract:

The fact that EBV infection is ubiquitous in the world but EBV associated malignant diseases incidence differs in geographic regions raises the possibility that some subtype of EBV is involved in some kind of tumor. We compare polymorphism of EBV among Epstein-Barr virus associated gastric carcinoma (EBVaGC) and healthy adults in Shandong, which belongs to north of eastern China. Tumor samples from 236 gastric carcinoma cases and throat washings (TWs) from 135 healthy adults were collected and screened for EBV genome. Then the 17 EBVaGCs and 33 EBV positive TWs were examined for type 1/2 EBV and polymorphism at BamHI F region, BamHI W1/I1 boundary region and XhoI restriction site in LMP1 gene. Among them, neither of type 2 or type f of BamHI F polymorphism was found in EBVaGC cases, nor type f in TWs. 25 of 33 TWs (75.8%) analyzed maintained type 1 virus, while 8 TWs (24.2%) maintained type 2 virus. Type I and i of BamHI W1/I1 polymorphism accounted 1(5.9%) and 16(94.1%) of EBVaGC and 11(33.3%) and 19(57.6%) of TWs, respectively. LMP1 XhoI(+) and (-) polymorphism accounted 0(0) and 15(88.2%) of EBVaGCs and 12(36.4%) and 18(54.5%) of TWs, respectively. The distribution rate of type i/XhoI(-) in EBVaGC(15/17, 88.2%) was higher than in TWs(5/33，15.2%). Our findings show that type 2 virus is rare in EBVaGC. Compared with relative data from southern China, type I and type f EBV strains are both to some extent geographically restricted polymorphism in China, with their higher detection rate in southern China than in northern China. A distinctive EBV strain (i/XhoI(-)) is associated with Chinese EBVaGC. It is interesting to find a candidate of specific tumor marker (XhoI (-)) for Chinese EBV related malignant diseases, as it was reported to be the predominant polymorphism in Chinese nasopharyngeal carcinoma and Hodgkin’s disease.

104 (RegID: 1292)

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CONSERVED MUTATION OF EPSTEIN-BARR VIRUS-ENCODED BARF1 GENE IN INDONESIAN NASOPHARYNGEAL CARCINOMA CASES.

Susanna H. Hutajulu, Servi JC Stevens, Sandra AWM Verkuijlen, Astrid E. Greijer, Sofia M. Haryana , Jaap M Middeldorp.

Posterabstract:

Background: BamHI-A rightward frame-1 (BARF1) is a potent carcinoma-specific EBV oncogene, but little is known about its genetic diversity in nasopharyngeal carcinoma (NPC).
Objective: This study aimed at defining the sequence of BARF1 in NPC compared to other EBV-related diseases. The correlation of BARF1 sequence diversity with viral load, EBV genotyping, and presence of antibodies to EBV was determined.
Method: Nasopharyngeal brushings and tissues from 56 NPC cases or blood from 15 patients with other EBV-driven disorders, and 7 spontaneous EBV positive B-cell lines (LCL) from healthy indonesian individuals were included. Purified DNA was analysed by PCR-direct sequencing. EBV-DNA viral load and EBV strain typing (based on EBNA2 sequence) were analyzed by PCR. Serology using IgA-[EBNA1/VCAp18] ELISA and IgG immunoblot analysis was done on all NPC sera.
Results: Most NPC isolates showed specific BARF1 nucleotide changes compared to prototype B95-8. At the protein level the mutations led to 3 main substitutions: V29A, W72G, H130R. Amino acid conversions were shown by 80.3% of NPC samples compared to 33.3% of non-NPC samples (p= 0.001). Amino acid changes did not associate with IgA ELISA titer (p= 0.495) and immunoblot diversity (p= 0.187). Almost all NPC and non-NPC samples contained EBV type 1. One NPC sample was mixed-type and 1 non-NPC sample was type 2. The viral load of either NPC or non-NPC samples was high, but not related to a particular BARF1 variant.
Conclusion: The EBV BARF1 gene shows genetic diversity which is more frequently present in NPC than in non-NPC EBV-related samples. These sequence variants may exist in the regional population as a predisposition of NPC. The functional implications for BARF1 mutations remain to be defined.

105 (RegID: 1294; 1296; 1297; 1298; 1299)

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A NOVEL 12P13.3 AMPLICON NASOPHARYNGEAL CARCINOMA

Grace Tin-Yun Chung, Yvonne Yan-Yan Or, Ka-Fai To, Siu-Wah Tsao, Timothy TT Yip, Pierre Busson, Kwok Wai Lo

Posterabstract:

Nasopharyngeal carcinoma (NPC) is a distinct type of head and neck cancer commonly occurring in Southern China. To decipher the molecular basis of this cancer, we performed high-resolution array CGH analysis on a panel of tumor lines and primary tumors to identify the genes involved in NPC tumorigenesis. In this study, multiple regions of gain were consistently found at 1q21.2-q21.3, 7q21.3, 11q13.1-13.3, 12p13, 19p13, 19q13. Importantly, a 2.1 Mb region at 12p13.31 was highly amplified in a NPC xenograft, xeno-2117. By FISH mapping, we further delineated the amplicon to a 1.24 region flanked by RP11-319E16 and RP11-433J6. Copy number gains of this amplicon were confirmed in 21/41 (51%) primary tumors while 3 cases (7.3%) showed high copy number amplification. Among the 13 genes within this amplicon, three candidate genes, LT-beta R (Lymphotoxin-beta receptor), TNFRSF1R (tumor necrosis factor receptor superfamily, member 1A) and FLJ1066 (PLEKHG6, pleckstrin homology domain containing, family G (with RhoGef domain) member 6) were specifically overexpressed in xeno-2117 having the 12p13.3 amplification. Of these three candidates, only the LT-beta R was proven to be frequently overexpressed in primary tumors. As a critical component of NF-kappaB signalling pathway, LT-beta R was recently reported to be able to transform mouse fibroblast cells. Importantly, aberrant NF-kappaB signal were found in all EBV-positive NPC tumors and play crucial role in NPC development. Overexpressing LT-beta R in a normal nasopharyngeal epithelial cell line NP69 resulted in an increase in NF-kappaB activity. Thus, amplification of LT-beta R may contribute to NPC tumorigenesis by activating NF-kappaB signal and its downstream genes constitutively. These findings implied that LT-beta R may be a potential NPC-associated oncogene within 12p13.3 amplicon.

106 (RegID: 1464)

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CONSERVED MUTATION OF EPSTEIN-BARR VIRUS-ENCODED BARF1 GENE IN INDONESIAN NASOPHARYNGEAL CARCINOMA CASES

Susanna H. Hutajulu, Servi JC Stevens, Sandra AWM Verkuijlen, Astrid E. Greijer, Sofia M. Haryana , Jaap M Middeldorp
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Posterabstract:

Background: BamHI-A rightward frame-1 (BARF1) is a potent carcinoma-specific EBV oncogene, but little is known about its genetic diversity in nasopharyngeal carcinoma (NPC).
Objective: This study aimed at defining the sequence of BARF1 in NPC compared to other EBV-related diseases. The correlation of BARF1 sequence diversity with viral load, EBV genotyping, and presence of antibodies to EBV was determined.
Method: Nasopharyngeal brushings and tissues from 56 NPC cases or blood from 15 patients with other EBV-driven disorders, and 7 spontaneous EBV positive B-cell lines (LCL) from healthy Indonesian individuals were included. Purified DNA was analysed by PCR-direct sequencing. EBV-DNA viral load and EBV strain typing (based on EBNA2 sequence) were analyzed by PCR. Serology using IgA-[EBNA1/VCAp18] ELISA and IgG immunoblot analysis was done on all NPC sera.
Results: Most NPC isolates showed specific BARF1 nucleotide changes compared to prototype B95-8. At the protein level the mutations led to 3 main substitutions: V29A, W72G, H130R. Amino acid conversions were shown by 80.3% of NPC samples compared to 33.3% of non-NPC samples ($p= 0.001$). Amino acid changes did not associate with IgA ELISA titer ($p= 0.495$) and immunoblot diversity ($p= 0.187$). Almost all NPC and non-NPC samples contained EBV type 1. One NPC sample was mixed-type and 1 non-NPC sample was type 2. The viral load of either NPC or non-NPC samples was high, but not related to a particular BARF1 variant.
Conclusion: The EBV BARF1 gene shows genetic diversity which is more frequently present in NPC than in non-NPC EBV-related samples. These sequence variants may exist in the regional population as a predisposition of NPC. The functional implications for BARF1 mutations remain to be defined.

107 (RegID: 1518)
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QUANTITATIVE PROFILING OF EBV MICRORNA EXPRESSION KINETICS IN AN IN-VITRO MODEL OF EBV TRANSFORMATION IN LYMPHOMA.

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Posterabstract：
Lymphomagenesis is a complex process which in part reflects the nature of the transforming event, as well as the stage of differentiation of the B-cell. Human models of B-cell differentiation and transformation are lacking. Current consensus supports the notion that EBV infects and activates nave B-cells, so that they undergo a germinal centre reaction that parallels the activation of a nave B-cell on exposure to an antigen. A well-characterized latency gene expression program drives this differentiation process. In some individuals, this process is interrupted by a transforming event resulting in a clonal proliferation of B-cells blocked at this differentiation state and latency profile.
MicroRNAs (miRNAs) are negative regulators of gene expression. EBV expresses at least 23 miRNAs in two clusters within the BART and BHRF1 regions of the genome. These miRNAs have been demonstrated to be differentially expressed by latency type in tumour cell-lines, suggesting distinct roles during EBV-driven B-cell differentiation and lymphogenesis.
The relationship between EBV miRNAs and the kinetics of B-cell differentiation and EBV latent gene expression has not been characterized. We hypothese that EBV miRNAs regulate EBV latency gene expression, B-cell differentiation and transformation. Using EBV infection and differentiation of isolated human B-cell subsets (nave and non-nave) as an in-vitro model of this process, the kinetics of EBV miRNA were correlated with latency gene expression using quantitative real-time RT- PCR .

108 (RegID: 1574; 1286)

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THE EVOLUTION OF EPSTEIN-BARR VIRUS INFERRED FROM THE POLYMORPHISMS OF THE GLYCOPROTEIN GP350/220 AND LMP1 GENES

Takeshi Sairenji1, Asako Kawaguchi1, 2, Kyosuke Kanai1, Yukio Satoh1, Chizu Touge2, Keiko Nagata1 and Yoshitsugu Inoue2

Posterabstract:

To study the epidemic variations of Epstein-Barr virus (EBV), we analyzed the nucleotide sequence of gp350/220 (BLLF1) gene for various cell lines and Japanese- wild isolates obtained from patients and healthy subjects using direct sequencing and compared the sequence to the prototype EBV, B95-8. The N-terminal region, which contains B-cell binding domains was highly conserved in most EBVs except for Jijoye, P3HR-1and a few wild isolates. The majority and the minority coincided with EBV types A and B, respectively and they were designated as types a and b. Type A/a was detected in most Japanese-derived cell lines and wild isolates, but type B/b was detected in only a few wild isolates. The former was all classified as China1 subtype with the LMP1 gene, whereas the latter was in the Med and China2 subtypes. The C-terminal region had more diversity than the N-terminal region and lacked the characteristic divergence between type A/a and type B/b. The phylogenic analyses of the gp350/220 and LMP1 genes suggest a mode of evolution of EBV into types A/a and B/b, and then to subtypes such as China1 and others.

109 (RegID: 1625; 1629)
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GENOME-WIDE QUANTITATIVE ANALYSIS OF EBV MICRORNAS IN NASOPHARYNGEAL CARCINOMA AND CELL LINES

SJ Chen YH Chen, GH Chen, WL Liao, KP Chang, YS Chang, and HC Chen

Posterabstract:

Epstein-Barr virus (EBV) is closely associated with human nasopharyngeal carcinoma (NPC). However, the role of EBV in the pathogenesis of NPC remains poorly understood. While several EBV-encoded proteins have been implicated in the oncogenic transformation of NPC, the limited expression of these EBV proteins suggest that other factors besides EBV-encoded proteins may also play a role in the development of EBV-associated NPC.

MicroRNAs (miRNAs) are a family of regulatory RNA molecules involved in a wide variety of cellular processes and play critical roles in tissue development, metabolism, immune responses as well as oncogenic transformation. These small ono-coding RNAs negatively regulate gene expression in a sequence-specific manner in species such as human, mouse, and C. elegans. MiRNAs have also been found on the genome of several human viruses, including EBV, KSHV, and HIV. Recent studies have uncovered a complex relationship between vertebrate virus and the miRNA pathway and reveal the roles of virus-encoded miRNAs in viral infection and virus-associated pathogenesis.

Currently, a total of 39 EBV miRNAs have been documented. Several EBV encoded miRNAs have been detected in NPC cell lines. To investigate the role of EBV miRNA in NPC development, we developed a robust multiplexed stem-loop RT-PCR method and conducted a genome-wide quantitative measurement to establish a comprehensive expression profile of EBV miRNAs in NPC tissues and cell lines. Some of the EBV miRNAs were expressed at levels higher or similar to the most abundant human miRNAs. The expression patterns of EBV miRNAs in NPC tissues closely resemble the expression profiles of EBV miRNAs in NPC cell lines. Unsupervised hierachical clustering analysis using global EBV miRNA expression profile clearly distinguished NPC tissues and NPC cell lines from EBV+ B cell lines. In NPC tumor samples, the expression levels of EBV miRNAs were positively correlated with the levels of EBER1, a well documented marker for EBV viral load. These results suggest that several EBV miRNAs may be use as additional markers for EBV detection. Studies are underway to investigate the potential roles on EBV miRNAs in NPC.

110 (RegID: 1719; 1720)

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EPSTEIN-BARR VIRUS ENCODED SMALL RNA ACTIVETES TOLL-LIKE RECEPTOR 3 SIGNALING

Dai Iwakiri, Mrinal Samanata, Kenzo Takada

Posterabstract:

Epstein-Barr virus-encoded small RNA (EBER) is non-coding RNA, which forms stem-loop structure by intermolecular base-pairing giving rise to double-stranded (dsRNA)-like molecule and exists abundantly in EBV-infected cells. Recently we have reported that EBER is recognized by retinoic acid-inducible gene (RIG)-I, a cytosolic sensor of virus-derived dsRNA, leading to activation of RIG-I signaling in EBV-infected cells. Here we identified that EBER is released from EBV-infected cells, leading to activation of signaling induced by toll-like receptor (TLR) 3, which is a sensor of extracellular viral dsRNA. Treatment of cells with in vitro-synthesized EBER caused induction of type I interferon (IFN) in a TLR3 dependent manner. A substantial amount of EBER, which was sufficient to activate TLR3 signaling, was released from various EBV-infected cells. Further study demonstrated that the majority of the released EBER exists as a complex with a cellular EBER-binding protein La, suggesting that EBER is released from the cells by active secretion of La. Sera from patients with infectious mononucleosis (IM), chronic active EBV infection (CAEBV) and EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH) contained EBER, and stimulation with RNA purified from the sera resulted in TLR3 activation in LCLs and PBMCs. Furthermore, DCs treated with EBER showed mature phenotype and antigen presentation capacity. These findings suggest that EBER, which is released from EBV-infected cells, is responsible for immune activation by EBV through TLR3 signaling. EBER-induced activation of innate immunity would account for immunopathologic diseases caused by active EBV infection.

111 (RegID: 1774)
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DISCORDANT EPSTEIN-BARR VIRUS (EBV) STRAINS IN PLASMA, PERIPHERAL BLOOD MONONUCLEAR CELLS AND SALIVA OF CHILDREN WITH PRIMARY EBV INFECTION

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Posterabstract:

Aim: Long term virus carriers harbor multiple Epstein-Barr virus (EBV) strains. How these strains are acquired during primary infection and how they distribute within the body compartments are not known. We ask (1) whether multiple EBV strains are acquired during primary infection, (2) whether these strains persist over time, and (3) how these strains distribute between B cell and epithelial cell pools.

Methods: Blood and saliva were collected from twenty Chinese children confirmed to have primary EBV infection serologically (twelve children had infectious mononucleosis and eight children had asymptomatic infection) at different time points from the time of diagnosis to one year post-infection. EBV strains contained in peripheral blood mononuclear cells (PBMC), plasma and saliva were characterized by a panel of fluorescent PCR assays on two polymorphic latent genes, EBNA2 and LMP1. Real-time PCR typing assay classified EBV strains into types 1 and 2 based on type-specific polymorphisms at the EBNA2 locus. LMP1 typing assay identified EBV strains with or without 30-bp deletion at the LMP1 locus. Fluorescent heteroduplex tracking assays (HTA) distinguished five EBNA2 subtypes (group 1, 2, 3a, 3b and 3e) and seven LMP1 strains (China 1, China 2, Med+, Med-, NC, Alaska and B95-8).

Results: No significant difference in EBV strain distribution was observed between infectious mononucleosis and asymptomatic infections. Type 1 virus was the dominant virus (found in 19 of 20 cases), mostly as single type infections (14 of 20 cases). EBNA2 groups 1 and 3e were predominant in PBMC and saliva whereas groups 1, 2, 3b and 3e were all found in plasma, often as multiple strains in the same timepoint. Co-infection of LMP1 deleted and non-deleted strains were detected in 75% (15 of 20) of cases whilst deleted strains only were found in the remaining 25% (5 of 20). LMP1 strains, China 1, China 2 and Med+, were the predominant strains which were frequently detected as multiple strains in PBMC, plasma and saliva in the same timepoint. Similar viral strains were found in PBMC and saliva but different or additional strains were often detected in plasma of the same patient in the same timepoint.

Conclusion: Multiple EBV strains are already acquired during primary infection and that these strains can persist over time. Different viral strains can be detected in PBMC, plasma and saliva at different timepoints. Concordance of viral strains in PBMC and saliva at the same timepoint is frequently observed. In contrast, partial or complete discordance of viral strains in plasma from those in PBMC and saliva is often detected, indicating that plasma likely contains viral strains released from multiple sources, including peripheral blood and epithelial cell pools as well as other cell compartments such as the lymphoepithelial and secondary lymphoid tissues.

112 (RegID: 1802)
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AN EBV INFECTED IN A NK/T CELL LINE HAS A 59.7-KILOBASE-PAIR DELETION WHICH INCLUDES BART MIRNAS AND MAJORITY OF LYTIC GENES

Se Mo Jun, Won Seog Kim, Norio Shimizu, Yasuaki Harabuchi and Suk Kyeong Lee

Posterabstract:
Epstein-Barr virus (EBV) is associated with variety of malignancies. MicroRNAs (miRNAs) are 21–24 nt long small noncoding RNAs. They are involved in post-transcriptional gene regulation by binding to 3'UTR of target mRNAs. EBV encodes two groups of viral miRNAs: BHRF1 and BART miRNAs. Previous reports show that BART miRNAs are expressed in all 3 latencies, while BHRF1 miRNAs are expressed only in latency III. However, the function of these viral miRNAs is not yet clear. In this study, we show that a latency II EBV-infected NK/T cell line contains a deletion between BamHI B and Nhet fragment, which involves an approximately 59.7 kbp region. This region encodes majority of lytic genes and entire BART miRNAs. Hence, none of the BART miRNA is expressed and the lytic cycle cannot be induced unlike the other NK/T cell lines. This naturally derived EBV infected NK/T cell line with entire BART miRNAs deletion may be useful for the functional study of EBV BART miRNAs.

113 (RegID: 1819)

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STRATEGIES TO CONTROL NASOPHARYNGEAL CARCINOMA: A 20 -YEAR CLINICAL & SEROLOGICAL PROSPECTIVE STUDY IN SOUTHERN CHINA.

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3 Department of Microbiology, and 4 Department of Clinical Oncology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, PRC.

Posterabstract：

A prospective study of NPC was conducted in southern China, in which 42048 healthy subjects were clinically and serologically followed up for 16 years and 171 cases of NPC detected during this period and 168 of these cases were further follow-up for 5 years.

The results showed that the serum EBV antibody level of 90% of the patients was raised and sustained at high levels for up to 10 years before diagnosis. The mean duration of this preclinical window was estimated to be 29 months. The latter was estimated as the interval between the time when the serologic change was first observed and when the tumor has undergone malignant changes to emerge as UICC stage I disease.

It was found that 60% (44/73) of cases detected by routine NP examination of the seropositive subjects were presented with stage I disease, showing no or a minimum of symptoms, while 93% (91/98) patients symptomatically presented in OPD had the more advanced stage II, III or IV disease. The 5 year actuarial disease-free survival and overall survival of patients with stage I disease were 90% and 93%, respectively, and their distant relapse rate was 2%. In contrast, the survival rates of the patients with the advanced disease were <60% and distant relapse rates range 17% to 27%.

It was concluded that the preclinical serologic window supports the contention that EBV is involved in malignant transformation of NPC and as such, EBV serology provides an objective means to monitor tumor progression during preclinical stage of the cancer development. The results confirmed that NPC can be cured by early diagnosis and treatment and that this can be achieved though routine NP examination during this serologic window. In addition it is possible, in the light of the present findings, that EBV specific intervention during this window period might arrest tumor progression.

114 (RegID: 1917; 1918; 1919; 1920)

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MICRORNA 155 REGULATION IN EBV POSITIVE LYMPHOMA CELL

Qinyan Yin, Xia Wang, Jane McBride, Claire Fewell & Erik K. Flemington

Posterabstract:

MicroRNAs are a class of small non-coding RNAs that play a role in the regulation of cellular signaling and developmental processes. A number of microRNAs have been shown to be either oncogenes or tumor suppressors. One of the most highly implicated oncogenic microRNAs is miR-155. In lymphocytes, miR-155 is induced by B cell receptor (BCR) engagement and we have previously shown that induction of miR-155 by BCR activation is mediated by the transcription factors, JunB and FosB which bind to a conserved AP-1 element in the miR-155 promoter. miR-155 is highly expressed in EBV infected cells exhibiting type III latency but not type I latency and here we show that 1) miR-155 expression is induced by infection of EBV negative cells and by 5-Aza C induced expression of type III latency genes in type I latency cells. 2) Inhibition of the ERK, JNK, or p38 pathways is not sufficient to block miR-155 expression whereas inhibition of NF-kB suppresses expression of miR-155 and AP-1 proteins in the type III latency cell lines, IB4 and X50-7. 3) EMSA and ChIP analysis shows that the AP-1 proteins JunB, JunD and ATF3 bind to the miR-155 promoter and activate miR-155 in EBV positive type III latency cells. 4) Reporter experiments and RT-PCR analysis of type I latency cells transduced with retroviruses expressing latency gene products indicate a role of LMP1 and LMP2A in the induction of miR-155. Nevertheless, our data indicate that LMP1 and LMP2A are insufficient to cause the high level expression of miR-155 that is typically observed in type III latency cells. In summary, AP-1 proteins induced by type III latency gene expression bind to the miR-155 promoter to regulate miR-155 expression in type III latency cells.

115 (RegID: 1944; 1943)

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PATHOGENESIS OF EBV BLLF1 GENE IN THE DEVELOPMENT OF LYMPHOMA IN TRANSGENIC MICE

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Posterabstract:

The Epstein-Barr virus (EBV) membrane antigen(MA) coded by EBV BLLF1 gene is a glycoprotein(gp350/220) which binds to the receptor CD21(CR2) on host cells such as B lymphocyte, T lymphocyte and epithelial cells initiating the infection of EBV. The binding itself can also lead to the activation of host cells and result in a serious of signaling pathways activation. As such, it is possible that this protein may play an important role in the pathogenesis of EBV related neoplasia. Despite this, less attention has been paid to this protein in the study of tumorigenicity of EBV; the reason may be due to the fact that this protein is mainly expressed in the active phase of EBV infection which lasts a short period of time mostly. However, the latent infection of EBV can become active repeatedly during the lifelong period, so the role of MA in the pathogenesis of EBV related diseases needs to be explored. The experiments described here were initiated to study the pathogenesis of this protein in vivo with a transgenic mice model carrying MA coding gene BLLF1. Transgene expression was demonstrated in tumor cells of two founder mice which developed lymphoma at the age of 4 and 8 months respectively. After more than 25 generations of breeding, the phenotype of lymphoma is found stable. To our knowledge, this is the first demonstration that BLLF1 gene is oncogenic in vivo suggesting that the gene product may play a direct role in the development of lymphoma and possibly other EBV-associated malignancies.

116 (RegID: 1988)
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LATENCY TYPE SPECIFIC DISTRIBUTION OF EPIGENETIC MARKS AT KEY LATENCY PROMOTERS OF EPSTEIN-BARR VIRUS IN TUMOR CELL LINES

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Posterabstract：
Epigenotypes differ in transcriptional activity despite their identical DNA sequence. We wished to characterize the distinct viral epigenotypes associated with EBV latency patterns of tumor cells. The unique NPC cell line C666-1 maintains latent EBV genomes during serial passages in vitro. Using high resolution methylation mapping, genomic footprinting, and chromatin immunoprecipitation, we examined the key latency promoters Qp, Cp, LMP2Ap and the EBER-promoters of EBV in C666-1 and lymphoid cell lines.
At silenced Cp, C666-1 cells showed a high level of CpG-methylation and inactived histones, but no specific transcription factor binding. Contrary, Qp directing EBNA 1 transcripts in latency class I, and the EBER-promoters were entirely unmethylated, and showed extensive transcription factor binding in all examined cells. Transcription factor binding at the EBER-promoters and their transcriptional activity could be abrogated by CpG-methylation. While Qp was unmethylated in all cells regardless of its activity status, only the active Q-promoter in C666-1 and in Burkitt´s Lymphoma cells was highly enriched in transcriptionally activated acetylated histones. At LMP2Ap, we found a high level methylation and enrichment of activated histones in C666-1 cells. DNA methylation may antagonize the effect of activated histones, resulting in a barely detectable LMP2A expression in this epithelial cell line.
We conclude that DNA methylation contributes to silencing of Cp not only in lymphoid, but also in C666-1 cells. Analysis of protein-DNA interactions at Qp and the EBER locus suggest that their regulation may be similar in lymphoid and epithelial cells. The oriP-EBER locus may function as a locus control region for the EBV genome not only in lymphoid, but also in epithelial cells. Work is in progress to rid NPC cells of the EBV genome via the locus control region. This is the first description of acetylation islands at EBV latency promoters in tumor cell lines.

NOTES:

Poster sesions

Session 4: Signal Transduction

117 (RegID: 1013)

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EBV-ENCODED LMP1 REGULATES OP18/STATHMIN SIGNALING PATHWAY BY CDC2 MEDIATION IN NASOPHARYNGEAL CARCINOMA CELLS

Xuechi Lin, Sufang Liu, Xiangjian Luo, Xiaoqian Ma, Lili Guo, Lili Li, Zijian Li， Yongguang Tao, Ya Cao※

Posterabstract:

Oncoprotein 18/stathmin (Op18/stathmin) plays a crucial role in maintaining cell biological characteristics by regulating microtubule dynamics, especially entry into mitosis; phosphorylated Op18/stathmin promotes microtubule polymerization to form the mitotic spindle, which is essential for chromosome segregation and cell division. Cdc2 is a critical kinase in starting M phase events in cell-cycle progression and is a positive regulator of the cell cycle. Latent membrane protein 1 (LMP1) is an Epstein-Barr virus (EBV)-encoded oncogenic protein that is able to induce tumorigenesis via various signaling pathways. This study focused on regulation by LMP1 of Op18/stathmin signaling in nasopharyngeal carcinoma (NPC) cells and showed that LMP1 regulates Op18/stathmin signaling through cdc2 mediation, LMP1 upregulates cdc2 kinase activity, and Op18/stathmin phosphorylation promotes the interaction of cdc2 with Op18/stathmin and microtubule polymerization during mitosis, and inhibition of LMP1 expression attenuates the interaction of cdc2 and Op18/stathmin and promotes microtubule depolymerization. These results reveal a new pathway via which LMP1 regulates Op18/stathmin signaling by cdc2 mediation; this new signaling pathway not only perfects the LMP1 regulation network but also elucidates the molecular mechanism of LMP1 that leads to tumorigenesis.

118 (RegID: 1048; 1049; 1050)

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THE NUCLEAR EXPORT OF THE EPSTEIN-BARR VIRAL PROTEIN EB2 IS MEDIATED BY DIRECT INTERACTION WITH THE CELLULAR FACTOR TAP/NXF1.

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Posterabstract:

The Epstein - Barr virus (EBV) early nuclear protein, EB2 (also called Mta, SM or BMLF1) promotes the nuclear export of a subset of early and late viral mRNAs and is essential for production of infectious EBV virions. A striking characteristic of mRNA export factors is that they shuttle between the cytoplasm and the nucleus. This shuttling is mediated by specific factors interacting with peptide motifs called nuclear export signals (NES) and nuclear localization signals (NLS). We had previously identified a novel CRM1-independent transferable NES and two NLS at the N-terminus of EB2 between aa 61 and 146.

In order to understand by which mechanisms EB2 shuttles, we have now purified protein complexes associated with the N-terminal part of EB2 (aa 1 to 183) by using a Tandem Affinity Purification system and have identified by mass spectrometry the cellular mRNA export factor TAP and the importin-β as components of these complexes. By confocal microscopy, we have shown that TAP and EB2 colocalize in the cell nucleus. Then, by the means of various interaction studies (co-immunoprecipitation assays and GST-pull down) we have found that TAP directly binds the EB2 NES (aa 61-146) in which TAP specifically interacts with a region rich in basic amino-acids. This region overlaps with one of the previously mapped NLS. Finally, titration of TAP by overexpression of the TAP-interacting domain of the 9G8 SR protein abolishes EB2's shuttling. Thus, EB2 appears to recruit TAP both via a direct interaction with a basic-rich domain localized in the N-terminal part of the protein and, as previously published, through an indirect interaction (via REF) with a central domain of EB2 (aa 213-236). However, only the direct recruitment of TAP on the N-terminal of EB2 appears to be critical for the shuttling of the protein.

119 (RegID: 1082; 1083)

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FUNCTIONAL DIFFERENCES OF TYPE 1 AND TYPE 2 EBNA2

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Posterabstract:

EBV strains are classified as type 1 or type 2 (A and B type) according to the sequence of the EBNA2 gene. Type 1 EBV immortalises B lymphocytes more efficiently than type 2 EBV and this difference was previously mapped to the EBNA2 locus. The protein sequences of type 1 and type 2 EBNA2 are only 55% identical. We previously developed a system that functionally distinguishes type 1 and type 2 EBNA2 using the ability of the EBNA2 allele to suppport proliferation of the EREB2.5 cell line (Lucchesi et al, 2008 J Virol 82: 7456-7466).

Although the parts of EBNA2 that differ most between the two types lie in the N terminal half of EBNA2, we have now shown that substitution of the C terminal third of the type 1 protein into the type 2 EBNA2 is sufficient to confer the type 1 phenotype in the EREB2.5 assay. Further more detailed mapping of the sequences that are responsible for this will be presented.

Differential regulation of cell genes such as CXCR7 and of the viral LMP1 gene correlates with the greater transforming activity of type 1 EBNA2. Current models of EBNA2 function (based on the study of type 1 EBNA2) have focussed on its interaction with RBP-Jk. The mechanism of differential gene regulation by the two EBNA2 types could include differences in the factors with which they interact at some promoters. We have now shown that transcription of the LMP1 promoter in its normal context in the viral episome is greater and more rapid in response to induction of type 1 EBNA2 activity than type 2 EBNA2 whereas the same EBNA2 alleles activate the LMP2A promoter equally. The results indicate functional differences in the mechanisms by which the EBNA2 types regulate genes important for cell proliferation and survival.

120 (RegID: 1085; 1086)
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NUCLEOSOME ASSEMBLY PROTEINS BIND TO EPSTEIN-BARR NUCLEAR ANTIGEN 1 (EBNA1) AND AFFECT ITS FUNCTIONS IN DNA REPLICATION AND TRANSCRIPTIONAL ACTIVATION

Shan Wang and Lori Frappier

Posterabstract:
EBNA1 is the only viral protein required for the persistence of Epstein-Barr virus (EBV) genomes through its contributions to the replication, mitotic segregation of EBV DNA, and transcriptional activation of other viral latency genes. Our laboratory has shown that the histone chaperones NAP-1, NAP-2 (Nucleosome assembly protein) and TAF-I (Template activating factor-I, including isoforms α andβ) bind to EBNA1 by affinity chromatography. Growing evidences have suggested that these histone chaperones are involved in the processes of chromatin assembly and disassembly which in turn regulate DNA transcription, replication and repair. Therefore we have examined the contributions of NAPs and TAF-I to EBNA1 functions in DNA replication and transcriptional activation. Co-immunoprecipitation experiments confirmed that NAP-1, NAP-2, and TAF-I interact with EBNA1 in EBV-positive Burkitt's lymphoma Raji cells. Glycerol gradient sedimentation assays with purified proteins demonstrated that both NAP-1 and TAF-Iβ were able to bind directly to EBNA1 and these interactions were shown to increase the solubility of EBNA1. The effects of NAP-1, NAP-2, and TAF-I on EBNA1 functions were examined by either over-expressing or silencing these proteins in nasopharyngeal carcinoma cells (CNE-2Z), which are also associated with EBV latent infection. Either silencing or over-expressing NAP-1, NAP-2, or TAF-I decreased EBNA1-dependent transactivation of the CAT reporter, suggesting that these proteins contributed to EBNA1-mediated transactivation through protein complex formation. Furthermore, silencing TAF-I stimulated replication, while over-expressing TAF-I decreased replication, indicating that TAF-I acts as a repressor of EBNA1 replication activity. In keeping with these findings, chromatin immunoprecipitation assays in Raji cells showed that NAP-1 preferentially localized to the EBV enhancer element FR; while TAF-I localized to both the FR and the origin of DNA replication (DS), both of which are bound by EBNA1. Therefore, nucleosome assembly proteins affect EBNA1-mediate transcriptional activation and replication through their interactions with EBNA1.

121 (RegID: 1105; 1106)

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EBV'S LATENT MEMBRANE PROTEIN 2A MIMICS BCR FUNCTIONS

Stephanie Medele, Liudmila Matskova, Dagmar Pich, Christoph Mancao, Wolfgang Hammerschmidt.

Posterabstract:

Hodgkin lymphoma, Burkitt lymphoma and post-transplantation lymphoma are associated with Epstein-Barr virus and originate from clonal germinal centre (GC) B cells. During the process of somatic hypermutation, GC B cells can acquire deleterious or other detrimental mutations in their immunoglobulin genes, which can result in a non-functional B-cell receptor (BCR) or even loss of surface immunoglobulin expression. In vivo these B cells are eliminated by immediate apoptosis as a rule. B cells latently infected with EBV express the viral latent membrane protein 2A (LMP2A), which gives BCR-negative B cells a selective advantage (Mancao and Hammerschmidt, Blood 2007, 110:3715-21). LMP2A and BCR are structural homologous because they both contain a characteristic ITAM domain. Therefore, LMP2A might mimic BCR signaling and provide BCR-low or BCR-negative B cells with a surrogate BCR-like signal to escape negative selection in GC reactions.

In contrast to the BCR, LMP2A signaling has not been analyzed systematically because the viral protein is constitutively active and independent of a ligand. Here we compare signaling activities and regulation of cellular genes induced by the BCR and/or LMP2A in EBV-infected B cells. Cells were infected with either wild-type EBV or viral mutants, which lack LMP2A or encode an inducible chimeric LMP2A molecule. We found that both BCR and LMP2A signal through Syk, BLNK, PLC-γ2, Akt, IKKß and Erk. In a microarray-based gene expression analysis many cellular genes show a very similar regulation by either BCR or LMP2A indicating that both receptors affect similar functions in B cells. Our results indicate that LMP2A can replace the BCR and explain why EBV-infected B cells, which express LMP2A, do not undergo apoptosis in the absence of a BCR-derived signal. Our findings imply an important role for EBV's LMP2A in the process of lymphomagenesis in EBV-positive B-cell lymphomas.

122 (RegID: 1113; 1127)
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THE IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIF OF EBV LMP2A PLAYS A ROLE IN LMP2A-INDUCED TRANSFORMATION

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Posterabstract:

Latent membrane protein 2A (LMP2A) of Epstein-Barr virus (EBV) is widely expressed in EBV-infected cells within the infected human host and EBV-associated malignancies, suggesting that LMP2A is important for EBV latency, persistence and EBV-associated tumorigenesis. We have shown that LMP2A provides not only an antiapoptotic signal but also transformation activity through the activation of phosphatidylinositol 3-kinase (PI3-K)/Akt pathway in a non-hematopoietic human gastric carcinoma cell line, HSC-39 cells. In this study, to clarify the mechanisms of LMP2A-mediated transformation activity, we focused on the immunoreceptor tyrosine-based activation motif (ITAM) within the cytoplasmic amino-terminal domain of LMP2A. ITAMs are signaling motifs associated with activation, survival and differentiation. For this aim, LMP2A and LMP2A ITAM mutant-expressing HSC-39 cells were generated. As we have shown previously, LMP2A induced Akt phsophorylation and anchorage independent cell growth in HSC-39 cells. However, these effects were abolished in LMP2A ITAM mutant-expressing HSC-39 cells. In addition, LMP2A co-localized and bound to Syk, resulting in the tyrosine phosphorylation of both LMP2A and Syk. This interaction was dependent on the LMP2A ITAM. Furthermore, the tyrosine kinase inhibitor Genistein and the Syk inhibitor piceatannol blocked LMP2A-mediated Akt phoshorylation and anchorage independent cell growth in HSC-39 cells. These results indicate that the interaction of LMP2A with Syk thorugh ITAM is a critical step for LMP2A-mediated transformation activity.

123 (RegID: 1118)

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EPSTEIN-BARR VIRUS ENCODED EBNA3 BINDS TO VITAMIN D RECEPTOR AND REPRESSES ITS TRANSACTIVATION FUNCTION

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Posterabstract:

EBV is one of the most highly transforming viruses known. The EBV-immortalized lymphoblastoid cell lines (LCLs) express six nuclear proteins (EBNAs) and three membrane proteins (LMPs), out of which five, EBNA-2, -3, -5, -6 and LMP1 are essential for transformation. The mechanism of transformation is not well understood, but it is clear that the virus exploits the normal signaling pathways of the B lymphocyte. We have discovered and published the seven previously unknown interactions between three transforming nuclear proteins, EBNA-3, EBNA-5 and EBNA-6 and cellular proteins, using the yeast two-hybrid system, GST-pull down assay and surface plasmon resonance (SPR).

Currently, we found 5 novel cellular binding partners of EBNA3 protein (DNA Helicase II, SWI/SNF1 chromatin remodeling complex, H2A, H2B, Heterogeneous Nuclear Ribonucleoprotein AB isoform A), based on immunoprecipitation studies and mass spectrometry. This study was done on various deletion mutants of EBNA3 protein (GFP fused at N-terminal region of EBNA3 mutants).

It is known that SWI/SNF1 (one of novel cellular binding partner) cooperates with VDR complex and regulates various responsive genes of VDR (Vitamin D receptor). We performed immunoprecipitation studies and showed that EBNA3 is one of the units of VDR protein complex. Moreover, using Real Time PCR, we showed that EBNA3 represses the expression of VDR responsive genes. This study was done by relatively comparing LCLs and EBNA3 negative LCLs. Upon the treatment with Vitamin D3 (VDR ligand) we observed similar repressive activity of EBNA3 on VDR responsive genes. In conclusion, we showed that EBNA3 interacts with VDR complex and represses its transactivation function.

124 (RegID: 1119; 1120)

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EPSTEIN-BARR VIRUS ENCODED LATENT MEMBRANE PROTEIN 1 MEDIATED SIGNALING TRANSDUCTION PATHWAY IN NASOPHARYNGEAL CARCINOMA

Ya Cao

Posterabstract:

Latent membrane protein 1(LMP1) is considered as oncogene among Epstein-Barr virus (EBV) encoded proteins. C terminal-region of LMP1 is the key functional domain which could trigger multiple signaling transduction cascades to alter cell growth and survival. Combined the novel strategy of phosphor-protein enrichment with proteomics technology, also called “signalomics”, twenty-five signaling molecules triggered by LMP1 in nasopharyngeal carcinoma (NPC) were identified. These new signaling molecules or targets show some unknown function in LMP1 mediated signaling pathways. According to the existent signaling pathway previously and these new targets, we constructed the whole signaling network triggered by LMP1 for the first time in NPC.

Based on the signaling transduction network mediated by LMP1, we systemic confirmed multiple kinase signaling pathways activated by LMP1, such as PI-PLC/PKC、MAPK、JAK/STAT and PI3K/AKT signaling pathways. These activated kinases could phosphorylate some important signaling molecules in network, including STAT3, Annexin A2, p53, EGFR, stathmin and survivin, which promoted cell malignant proliferation and the inhibition of apoptosis. Besides these “old”, “nodel” molecules embedded with new function in network, some new proteins in LMP1 mediated signaling transduction network have been identified in success, such as Annexin A2 and stathmin. Our data showed that LMP1 increased the serine phosphorylation of Annexin A2 by activating the PI-PLC-PKCα/PKCβ pathway, especially by PKCβ pathway. And serine 25 phosphorylation of annexin A2 was associated with the nuclear entry of annexin A2 and cell proliferation. LMP1 could regulate the phosphorylation of stathmin through cdc2 and MAPK signaling pathway with cell-cycle phase. So there should be more new proteins and their new function need to be confirmed to enrich the LMP1 mediated signaling network. These findings provide us with a novel view of comprehensive understanding the function of LMP1 from the signaling transduction in the carcinogenesis of NPC.

125 (RegID: 1121)

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THE FUNCTIONAL ACTIVATION OF P53 REGULATED BY EPSTEIN-BARR VIRUS ENCODED BY LATENE MEMBRANE PROTEIN 1

Lili Li

Posterabstract:

EBV encoded latent memebrane protein 1 (LMP1), an oncogenic protein, plays an important role in the carcinogenesis of nasopharyngeal carcinoma (NPC). Unlike most human tumors, nearly 100% are wild-type p53 in NPC. P53 accumulation is significantly correlated to LMP1 in NPC biopsies. However the molecular mechanisms leading to the stabilization and functional activation of p53 mediated by LMP1 have not been completely elucidated. Here, we present the first evidence that LMP1 could promote wt-p53 nuclear accumulation, up-regulate transcriptional activity and protein expression of p53. Interestingly, p53 can be activated and phosphorylated clearly at Ser15, Ser20, Ser392 and Thr81 mediated by LMP1. MAP kinases play a critical role in LMP1-induced phosphorylation of p53 at multiple sites, and JNK signaling pathway mediated p53 Ser 20 phosphorylation is more important than others. Furthermore, the phosphorylation of p53 enhanced the expression of p21, MDM2. Interestingly, we found that LMP1 decreased the binding of MDM2 to p53, so it suggested that LMP1 could regulate p53 function via ubiquitination, moreover the ubiquitination of p53 regulated by LMP1 is phosphorylation depended. When inhibited p53, resistance to apoptosis was observed. Taken together, these results suggest that accumulated p53, as an important transcriptional factor, play a key role in maintaining EBV latent infection, promote cell transformation and regulate of cell cycle in the carcinogenesis of NPC.

126 (RegID: 1152)
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THE LATENT MEMBRANE PROTEIN-1 OF EBV PROMOTES CELL GROWTH AND MIGRATION THROUGH ACTIVATION OF AKT, NF-kB AND DOWN-REGULATION OF PLAKOGLOBIN

Kathy H.Y Shair, Caroline Schnegg and Nancy Raab-Traub.

Posterabstract:
Latent membrane protein 1 (LMP1) is considered the major oncoprotein of EBV and is frequently expressed in nasopharyngeal carcinomas (NPC). LMP1 activates multiple signaling pathways including PI3K/Akt, NF-kB, JNK, p38, and ERK of which PI3K/Akt and ERK signaling are required for LMP1-induced transformation of rodent fibroblasts. C666-1 is the only cell line established from NPC that has retained the EBV episome. LMP1 is expressed at very low levels in these cells, however, exogenous expression of LMP1 enhances growth and induces migration of C666 cells. To identify the signaling pathways required for these growth effects, LMP1 and deletion mutants were expressed in C666 cells. LMP1-enhanced growth, as assayed by soft agar colony formation and MTS activity, required both of its signaling domains, CTAR1 and CTAR2. Activation of PI3K/Akt and NF-kB signaling by full-length LMP1 were both required and chemical inhibitors of these pathways blocked these effects. The requirement for the canonical NFkB pathway was confirmed using an IkBa (SS32/36AA) super-repressor. The potential metastatic properties induced by LMP1 were assessed using a transwell migration assay. Full-length LMP1 enhanced migration and this effect also required PI3K/Akt and canonical NFkB signaling. The LMP1-enhanced migration was linked to the transcriptional down-regulation of plakoglobin in that migration was impaired by restoring plakoglobin expression. Plakoglobin is a junctional protein found at adherens junctions and desmosomes and can also serve as a transcription factor that regulates Tcf/Lef-mediated transcription. Assessment of adherens junction and desmosome assembly, Tcf/Lef activated transcription, and the components of the transcription complexes indicated that the plakoglobin-mediated inhibition of migration was due to junctional loss of plakoglobin and not to transcriptional effects. These findings reveal that the profound effects of LMP1 on epithelial cell growth and migration require activation of PI3K/Akt and NFkB signaling. The finding that LMP1 induces a loss of plakoglobin that results in enhanced migration reveals that LMP1 has multiple effects on cell-adhesion and growth that contributes to EBV-induced oncogenesis.

127 (RegID: 1174; 1175)

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TUMOR SUPPRESSOR DELETED IN LIVER CANCER-1 (DLC-1) GENE TRIGGERS APOPTOSIS IN NPC CELLS BY PERTURBING MITOCHONRIA (MI)

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Posterabstract:

DLC-1 is a bona fide tumor suppressor gene (TSG) mapped to chromosome 8p22-p21, a region frequently deleted in human malignancies. DLC-1 gene encodes a GTPase activating protein (GAP), which negatively regulates the activity of Ras homolog (Rho) family. RhoGAP activity of DLC-1 plays an important role in regulating cell motility and suppressing cancer metastasis. Genetic and epigenetic inactivation of DLC1 is frequently found in human cancers, including nasopharyngeal carcinoma (NPC) and hepatocellular carcinoma (HCC), two major endemic tumors in southern China. The anti-proliferative effect of DLC-1 is evidenced with its inhibition of colony formation in NPC cells. The pro-apoptotic function of DLC-1 has also been suggested, but the underlying mechanisms remain unanswered. Since the human DLC-1 ortholog, DLC-2 was found to be localized on MI membrane; DLC-1 has been hypothesized to play a role in MI dependent apoptosis. To test this hypothesis, NPC cells were transfected with EGFP-tagged DLC-1 plasmid, and stained with tetramethylrhodamine ethyl ester (TMRE). Microscopically, DLC-1 signal was found to localize in cytoplasm and was significantly overlapped with TMRE signal. In addition, ectopic expression of DLC-1 dropped MI membrane potential (MMP) as evidenced by flow cytometry-based TMRE staining. Furthermore, we demonstrated that blocking of death receptor pathway（DR） by dominant negative FADD failed to suppress DLC-1 mediated MMP changes, suggesting that DLC-1 induced apoptosis was independent to DR pathway. We will next evaluate the effect of blocking stress-induced pathway with specific inhibitors on DLC-1 induced apoptosis. In summary, our results suggest that DLC-1 protein may target to MI membrane and induce apoptosis. The re-expression of DLC-1 is of mild pro-apoptotic activity; and thus it prompted us to test whether DLC-1 would potentiate apoptosis induced by the challenge of genotoxins and death ligands, and our study may provide insights to design of novel therapeutic modality for cancers.

128 (RegID: 1191; 1192)

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THE LMP1-INDUCED JNK PATHWAY CONTROLS CELL CYCLE PROGRESSION AND IS A POTENTIAL THERAPEUTIC TARGET FOR TUMOR THERAPY

Helmut Kutz, Gilbert Reisbach, Arnd Kieser

Posterabstract:

The latent membrane protein 1 (LMP1) of Epstein-Barr virus is essential for B-cell transformation by the virus. It is expressed in many EBV-associated malignancies such as Hodgkin's lymphoma, nasopharyngeal carcinoma or post-transplant lymphoproliferative disorders. LMP1 activates NF-kappaB, PI3-kinase, MAPK and c-Jun N-terminal kinase (JNK) signaling. Here, we investigated the functional role of the LMP1-induced JNK pathway in B-cell transformation. Inducible expression of the novel dominant-negative JNK1 (D169A) mutant, which lacks kinase activity, caused a proliferative block in LMP1-transformed human B-cells. Moreover, two chemically unrelated small molecule inhibitors of JNK efficiently interfered with proliferation of lymphoblastoid cells (LCLs), albeit without inducing significant apoptosis. Inhibition of the LMP1-induced JNK pathway in LCLs caused the downregulation of CDC2 (also named CDK1) expression, the essential G2/M cell cycle kinase, which was accompanied by a cell cycle arrest at G2/M phase transition. Taken together, these data demonstrated that the JNK pathway is essential for proliferation of LCLs, driving the cells through G2/M phase of the cell cycle by maintaining CDC2 expression. Next, we tested whether inhibition of JNK signaling is sufficient to block LCL growth in vivo. We found that the JNK-selective inhibitor SP600125 strongly retarded tumor growth of human EBV-transformed B cells in a xenograft model in SCID mice. Interestingly, this inhibitor showed massive effects on the tumors without any detectable systemic toxicity. In summary, our data support a critical role of the LMP1-induced JNK pathway for the proliferation of LMP1-transformed B-cells and characterize JNK as a potential therapeutic target for the intervention against EBV-induced malignancies.

129 (RegID: 1198; 1200; 1223; 1224)

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EBNA 3C-MEDIATED G2/M CHECKPOINT DISRUPTION THROUGH RGC-32 UPREGULATION

Sandra N. Schlick, Helen M. Webb, Claire M. Banwell, Andrea Gunnell and Michelle J. West

Posterabstract:

EBNA 3C plays a crucial role in the immortalisation of B-cells by EBV and promotes inappropriate progression through the G1/S, G2/M and M-phase checkpoints through multiple potential mechanisms.

We have identified the CDK1 activator, response gene to complement 32 (RGC-32), as the first cell-cycle regulator to be upregulated in EBNA 3C-expressing cells. RGC-32 overexpression has been detected in a number of cancer tissues and the EBV-mediated upregulation described here could therefore contribute to the development of EBV associated malignancies.

Using Affymetrix cDNA array and real-time PCR analysis we found specific upregulation of RGC-32 by up to 14-fold in EBNA 3C-expressing BJAB cell-lines. EBNA 3C-positive cell-lines with upregulated RGC-32 were able to override DNA damage-induced G2 arrest following exposure to etoposide or ionising radiation. Whereas control cells accumulated in G2/M following DNA damage, we found reduced accumulation of EBNA 3C-positive cells in G2/M and an up to 4.6-fold increase in the proportion of these cells progressing through the G2/M checkpoint into G1.

Significantly, we have provided the first demonstration that overexpression of RGC-32 alone is sufficient to perturb G2 arrest indicating that RGC-32 may play a role in G2/M checkpoint disruption by EBNA 3C via CDK1 activation. In support of this hypothesis we confirmed that recombinant RGC-32 increased CDK1 activity up to 2.5-fold in vitro. Surprisingly, we found that knock-down of RGC-32 increased the proportion of EBNA3C-expressing cells in G1. These results are consistent with previous reports of a role for RGC-32 in stimulating progression from G1 into S phase.

EBNA 3C activated the RGC-32 promoter by up to 2-fold in reporter assays indicating that at least part of the mechanism of upregulation of RGC-32 involves transcriptional activation.

Our results therefore identify RGC-32 as a novel regulator likely to be involved in the mechanism of cell-cycle disruption by EBNA 3C.

130 (RegID: 1210; 1211)

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DECOY RECEPTOR 3, ENHANCED BY EPSTEIN-BARR VIRUS LMP1, PROMOTES METASTASIS OF NASOPHARYGEAL CARCINOMA

Cheng-Hsun Ho, Chang-Hsuan Chiang, Pei-Fan Fong, Muh-Hwa Yang, C Chen, and Chi-Ju Chen

Posterabstract:

Both Epstein-Barr virus (EBV), a γ-herpesvirus, and decoy receptor 3 (DcR3), a soluble receptor belonging to tumor necrosis receptor (TNFR) superfamily, have been implicated in development of human malignancies. An earlier study showed that DcR3 expression is higher in EBV-positive lymphomas, suggesting EBV infection may enhance DcR3 expression. We consequently demonstrated EBV transcription activator, Rta, can upregulate DcR3 expression by binding to its promoter. However, the effect of EBV latent genes on DcR3 expression was unidentified. We demonstrated here EBV latent infection with LMP1 expression increased DcR3 expression level. Knocking down expression of EBV-encoded oncogenic gene, latent membrane protein 1 (LMP1), diminished DcR3 upregulation by EBV infection, indicating LMP1 is responsible for the activation. We found LMP1-induced DcR3 expression depended on both CTAR1 and CTAR2 and was mainly through NF-kB signaling events. We showed co-expression of DcR3 and LMP1 in NPC biopsies specimen. DcR3 levels in metastatic NPC is higher that in primary ones, suggesting the role of DcR3 in promoting NPC metastasis. Data herein suggest that LMP1 may contribute to metastasis and survival of EBV-infected cell by activation an immunomodulatory factor, DcR3, which prevents infected-cell detection by the host immune system and may enhance cell invasiveness.

131 (RegID: 1215; 1216; 1217; 1218; 1219; 1220)

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UPREGULATION OF ATF5 SUPPRESSES SAP EXPRESSION TO ACTIVATE T CELLS IN HEMOPHAGOCYTIC SYNDROME ASSOCIATED WITH EBV INFECTION AND IMMUNE DISORDERS

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Posterabstract:

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Hemophagocytic syndrome (HPS) is a fatal pro-inflammatory cytokine disorder associated with virus infections and immune disorders. Previously, we demonstrated that EBV latent membrane protein-1 (LMP-1) could downregulate the SAP gene, enhancing Th1 cytokine secretion in T cells, leading to HPS. The exact mechanism of this regulation of the SAP gene by LMP-1 remains to be clarified. In this study, using cDNA microarray, we identified that ATF5 was the candidate transcriptional repressor for SAP expression in LMP-1-expressed T cells. LMP-1 upregulated ATF5 via TRAF2,5/NFkappaB signals to suppress the SAP gene. Reporter assays and EMSA revealed that ATF5 bound differentially to two sites of the SAP promoter. In resting T cells, ATF5 bound predominantly to the high affinity site at the -81~ -74 region, while ATF5 additionally bound to the low affinity site at -305~ -296 in LMP-1-expressed T cells and subsequently disrupted the transcription of the SAP gene. In parallel, the Th1 cytokine secretion was enhanced. The same phenomenon was also observed in conditions such as ATF5 overexpression, phytohemagglutinin stimulation of primary T cells, or ligand-engaging of T cell lines. Therefore, the downregulation of the SAP gene by ATF5 may represent a common mechanism for the pathogenesis of HPS associated with either EBV infection or immune disorders with dysregulated T cell activation.

132 (RegID: 1230; 1231)

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LMP2A MODULATES SIGNALLING FROM TOLL-LIKE RECEPTOR (TLR)-3 AND TLR-4 IN HUMAN EPITHELIAL CELLS

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Posterabstract:

The EBV-encoded latent membrane protein, LMP2A, although not essential for viral transformation, plays a key role in promoting cell survival and viral latency. Although frequently expressed in EBV-associated carcinomas, the exact role that LMP2A plays in the maintenance of the transformed state remains to be elucidated. Previous studies from our group have uncovered a novel role for LMP2A and LMP2B in epithelial cells, namely, an ability to modulate signalling from type I and type II interferon receptors. These studies have now been extended to examine the effects of LMP2A and LMP2B on activity of the Toll-Like Receptor (TLR) family, focusing on those involved in viral immune responses (TLR 3, 4, 7 and 9). Both immunofluorescence staining and flow cytometery have established that TLR expression is significantly reduced in LMP2A but not in LMP2B-expressing cells. Furthermore, when stimulated with polyI:C or LPS, agonists which stimulate TLR3 and TLR4 respectively, LMP2A-expressing cells appeared almost refractory to agonist stimulation as assessed by interferon-b promoter luciferase reporter activity. Although LMP2B failed to attenuate TLR-mediated activation of IFN-b reporter constructs, both LMP2A and LMP2B were capable of modulating the induction of IFN-stimulated genes in response to poly(I:C) stimulation. These findings show that LMP2A and LMP2B target multiple effector pathways involved in innate immune recognition, and that situations may exist during epithelial cell infection where EBV is required to target one or both of these pathways to achieve stable persistent infection.

133 (RegID: 1232; 1233)

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IDENTIFICATION OF THE MECHANISM(S) CONTROLLING EBER1/2 RNA EXPRESSION IN EBV-INFECTED CELLS – A ROLE FOR EBNA1

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Posterabstract:

The EBV-encoded EBER genes are small, untranslated RNAs that are expressed in all forms of EBV latency and are implicated in EBV-driven oncogenisis. Although the EBERs are subject to regulation by RNA polymerase III (polIII), relatively little is known about their regulation by other cellular transcription factors and how EBV can induce high level expression (107 copies) in latently infected cells. The mechanisms of transcriptional regulation of the EBERs form the focus of our research.

During primary infection of B-lymphocytes, EBER expression occurs after expression of the EBNAs and LMPs. One hypothesis is another EBV latent gene product may regulate EBER1/2 expression. As previous findings have uncovered a role for trans-acting factors in controlling transcription of the EBERs, we sought to identify the EBV-encoded protein responsible for this effect.

Our previous findings have shown that EBV enhances EBER expression through induction of specific transcription factors. Transcriptional profiling and subsequent validation of EBNA1-expressing cells revealed that EBNA1 alone was sufficient to induce expression of transcription factors whose activity may impact on EBER1/2 expression. The generality of these effects was confirmed as EBNA1 was shown to elevate general polIII transcription in a number of epithelial cell lines. Furthermore, IHC staining revealed increased expression of TFIIIC subunits and p-ATF2 in NPC tumour biopsies.

To gain an insight into the mechanism by which EBNA1 achieves this effect we performed chromatin immunoprecipitation (ChIP) analysis on EBNA1 transfected and EBV-infected cells. Using this approach we demonstrate that EBNA1 is present at promoter elements of ATF2 and c-myc and suggest that this is the mechanism by which EBNA1 induces their expression. Future work will confirm whether this is also the case for TFIIIC. The data offer novel insights not only into the transcriptional regulation of the EBERs, but also the pleiotropic roles of EBNA1 in late
infection.

134 (RegID: 1235)
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THE EPSTEIN-BARR VIRUS (EBV)-ENCODED LMP2A AND LMP2B PROTEINS MODULATE INTERFERON SIGNALLING IN HUMAN EPITHELIAL CELLS.

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Posterabstract：

Whilst the association of Epstein-Barr virus (EBV) with the development of certain carcinomas is well documented, the precise role that individual EBV gene products play in the carcinogenic process remains unknown. The EBV-encoded latent membrane 2A (LMP2A) and LMP2B proteins are frequently expressed in EBV-positive carcinomas (NPC and gastric adenocarcinoma), suggesting that they play an essential role in epithelial cell growth transformation or in the maintenance of the virus-transformed state. Our recent findings have uncovered a novel function for these proteins in epithelial cells, namely, their ability to modulate signalling from type I and type II interferon receptors. We found that LMP2A/2B-expressing epithelial cells showed decreased responsiveness to interferon (IFN- and IFN-) as assessed by STAT1 phosphorylation, ISGF3 and GAF-mediated binding to ISRE and GAS elements, and ISRE/GAS luciferase reporter activation. Microarray profiling and Q-PCR validation showed that the effect of LMP2A and LMP2B on interferon-stimulated gene expression was “global”, with both proteins attenuating the induction of a many IFN-stimulated target genes. Although the levels of cell surface interferon receptors were similar between control, LMP2A and LMP2B-expressing cells, we found that the rates of interferon receptor degradation were significantly increased in the presence of LMP2A and LMP2B. Further analysis revealed that this function was attributed to increased endocytosis and lysosomal degradation of IFNRs. Preliminary investigations have uncovered a role for endosome acidification in this process as chloroquine, an endosomal acidification inhibitor, blocked the effects of LMP2A and LMP2B on IFNR degradation. In vivo monitoring of endosome acidification with LysosensorTM revealed increased kinetics of endosome acidification in LMP2A and LMP2B-expressing cells suggesting that LMP2A and LMP2B either function as proton (H+) pumps or modulate the activity of vacuolar ATPases.

135 (RegID: 1246; 1247)

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LMP1 INDUCES AN EPITHELIAL MESENCHYMAL TRANSITION (EMT) IN MDCK EPITHELIAL CELLS – KEY ROLES FOR ERK-MAPK, PI3-KINASE AND TYROSINE KINASES

L.Laverick, M.A.Morris, W.Wei, C.Woodman, L.S.Young and C.W.Dawson

Posterabstract:

Previous studies have shown that LMP1 can induce profound effects on the growth and differentiation of epithelial cells. LMP1 expression is associated with altered cell morphology, a loss of E-cadherin expression, and the acquisition of a more motile phenotype – all features of the epithelial mesenchymal transition (EMT). We have found that expression of LMP1 in MDCK cells is associated with EMT induction as evidenced by characteristic morphological changes and by accompanying loss of E-cadherin, desmosomal cadherin and tight junction protein expression. Biochemical profiling of cells for known signalling pathways implicated in EMT identified increased expression of TGFbeta and elevated ERK-MAPK and tyrosine kinase signalling in LMP1 expressing cells. RT-PCR and immunoblotting analysis established that LMP1 expression was associated with the induction of SLUG and TWIST, transcriptional repressors that target and negatively regulate expression of E-cadherin and desmosomal cadherins. The induction of EMT phenotype required a functional CTAR1 domain, as an LMP1 mutant defective for CTAR1 but carrying a functional CTAR2 domain, was unable to induce an EMT. Chemical inhibition of signalling pathways implicated in the induction of the EMT phenotype revealed contributions from ERK-MAPK, PI3-Kinase and tyrosine kinases, but not NF-κB, JNK or p38. Although LMP1 induced expression of a number of TGFbeta isoforms, inhibition of the TGFbeta signalling pathway with SB431542 did not reverse the EMT phenotype, ruling out contributions from the TGFbeta signalling pathway to LMP1-mediated EMT.

136 (RegID: 1266; 1267)

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THE DEUBIQUITINATING ENZYME A20 NEGATIVELY REGULATES LMP1 ACTIVATION OF IRF7

Shunbin Ning, Joseph S. Pagano

Posterabstract:

The deubiquitinating enzyme (DUB) A20 is an anti-apoptotic protein which is induced by the EBV oncoprotein LMP1, but its role in EBV oncogenesis has not been investigated. A20 is a member of the ovarian tumor (OTU) DUB family, and its DUB activity is required for termination of TLR signaling leading to NF-κB activation, and for blockage of TNF-induced cytotoxicity and apoptosis. IRF7 has oncogenic properties, and we have recently shown that LMP1 activates IRF7 through K63-linked ubiquitination which requires RIP and TRAF6 (Huye/Ning et al. Mol. Cell. Biol, 2007; Ning et al. Mol. Cell. Biol., 2008).

In this study, we show that A20 negatively regulates IRF7 transcriptional activity induced by LMP1. Deletion or mutation of A20 C-terminal Zinc finger motifs had no effect on the inhibition of IRF7 activity, whereas deletion of the N-terminal OTU domain ablated the ability of A20 to inhibit IRF7. Correspondingly, A20 N-terminus but not C-terminus interacts physically with IRF7. Moreover, A20 interacts with IRF7 endogenously in EBV latently infected type 3 cells in which expression of both A20 and IRF7 are constitutively induced by the high level of endogenous LMP1. Knockdown of endogenous A20 in Raji cells by expression of A20 shRNA vectors increases endogenous IRF7 activity and ubiquitination. In vitro deubiquitination assay results show that IRF7 is a substrate for the DUB A20 which removes K63-linked polyubiquitin chains from IRF7. Thus, A20 acts as a DUB to negatively regulate LMP1-stimulated IRF7 activity, and may participate in regulation of LMP1 oncogenic mechanisms at least through regulation of IRF7 activity.

137 (RegID: 1284; 1285)

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EPSTEIN-BARR VIRUS REPLICATION AND TRANSCRIPTION ACTIVATOR, RTA/BRLF1, INDUCES CELLULAR SENESCENCE IN EPITHELIAL CELLS

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Posterabstract:

Epstein - Barr virus (EBV) replication and transcription activator (Rta/BRLF1) is an immediate-early transcription factor that controls the conversion of a latent genome into lytic replication. By using doxycycline-inducible expression system, the present study demonstrates that EBV Rta efficiently elicits cellular senescence in epithelials. In the cells arrested by EBV Rta the expressions of p21, p27 and cyclin E were increased. In contrast, the concentration of cyclin D1, CDK4 and CDK6 were sharply decreased. Host cell DNA damage response (DDR) including the increasing phosphorylations of H2AX and p53 Ser15 were observed on day 3 and day 5 after EBV Rta expression, respectively. Finally, EBV Rta arrested cells exhibited strong senescence-associated -galactosidase staining on day 10 after doxycycline induction. Together, these results indicate that in addition to trigger the viral lytic replication, EBV Rta may concurrently initiates cellular senescence program that was previously undocumented. This finding thus provides new perspectives in understanding EBV pathogenesis.

138 (RegID: 1460; 1463)
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INTERPLAY BETWEEN EBV AND TELOMERE REGULATION

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Posterabstract:

Recent studies have shown that Origin Recognition Complex (ORC) binds to Epstein-Barr virus origin of plasmid replication (OriP). We have shown that the telomere repeat factor (TRF2) binds to OriP and facilitates ORC recruitment to OriP and enhances OriP replication and plasmid maintenance activity. We now report that TRF2 and EBNA1 use RNA to mediate interactions with ORC. Both EBNA1 and TRF2 recruit ORC through an RGG-like motif that was found binding to G-rich RNA capable of forming G-quadruplex structures. Recently, telomeres were found to encode telomere repeat-containing G-rich RNA, termed as TERRA, which can form G-quadruplex structure. We present new data demonstrating that both EBNA1 and TRF2 can bind TERRA RNA. Furthermore, we show that RNA binding contributes to ORC recruitment, DNA replication, and plasmid maintenance function. The cellular role of TERRA was explored using siRNA depletion. Loss of TERRA RNA reduces ORC recruitment at cellular telomeres and promotes telomere dysfunction, including telomere fusions and duplications. Interestingly, we found that TERRA RNA levels are altered during EBV immortalization of primary B-cells. This down regulation appears to correlate with an increase in telomerase activity and telomerase-associated regulatory factors. We propose that TERRA RNA regulation and telomerase activity play an intimate role in the establishment of stable latent infection and immortalization of primary B-lymphocytes.

139 (RegID: 1465; 1505)

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EBV RTA INDUCES A P21-DEPENDENT G1 CELL CYCLE ARREST

Sheng-Yen Huang;Tsuey-Ying Hsu;Jen-Yang Chen

Posterabstract:

There are ample evidences showing that regulation of the cell cycle is one strategy often used by viruses to create a more favorable environment for viral replication. The G1 phase delay or arrest could give the virus sufficient time to complete transcription and translation of viral genes and prevent the competition with host cellular DNA synthesis. This event usually occurs in the first stage of the lytic cycle and involves immediate-early or/and early proteins. Epstein-Barr virus (EBV) has both latent and lytic cycle. In the lytic stage, the virus expresses two immediate-early proteins, Zta and Rta. EBV Zta are known to bind p53 and stabilize it to enhance the accumulation of p21 protein, resulting in cell cycle arrest at the G1 phase. The role of Rta in the cell cycle regulation has not been fully addressed. In this study, we demonstrated that EBV Rta also has the ability to block cell cycle at the G1 phase through regulating the expression of p21 protein.

140 (RegID: 1472)

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ABERRANT ACTIVATING OF NOTCH SIGNALING IN EBV-POSITIVE NASOPHARYNGEAL CARCINOMA

Cheuk-Him Man, Samantha Wei-Man Lun, Jan Wai-Ying Hui, Ka-Fai To, Siu-Wah Tsao, Pierre Busson, Kwok-Wai Lo

Posterabstract:

Nasopharyngeal carcinoma (NPC) is an EBV-associated epithelial malignancy commonly found in Southeast Asia and Southern China. Despite multiple genetic and epigenetic changes has been identified, few aberrant signaling pathways involved in NPC oncogenesis were well characterized. In current study, we have comprehensively investigated the NOTCH signaling which is commonly involved in multiple oncogenic processes, such as differentiation, proliferation, apoptosis and angiogenesis, in six of EBV-positive NPC cell lines and xenografts. By quantitative RT-PCR and Western blotting, the expression of NOTCH components including ligands, receptors, regulators and effecors were examined. The two ligands, Jagged 1 (JAG1) and Delta-like 4 (DLL4), were overexpressed in all NPC tumors and a reciprocal relationship was shown. Accumulation of the intracellular domain of Notch3 (NICD3) was detected in all EBV-positive NPCs and only 50% of tumors showed NICD1 or NICD4 overexpression. Interestingly, expression of NUMB, a negative regulator of the NOTCH pathway was commonly found in these tumors. Among the common NOTCH effectors, upregulation of HEY1 and HEY2 were consistently found in the tumors. High frequency of JAG1, NICDs and HEY1 overexpression were further confirmed in a panel of primary tumors by immunohistochemical staining. No significant correlation between the expression of NOTCH components and EBV latent proteins (LMP1 and LMP2A) was observed. However, ectopic expression of LMP2A was able to induce the expression of multiple NOTCH receptors (NOTCH1 and NOTCH2) in nasopharyngeal epithelial cells (NP69). Our study demonstrated the constitutive activation of NOTCH signaling in NPC cell due to aberrant expression of multiple components in the pathway. Furthermore, we showed that treatment of gamma secretase inhibitor (GSI) and siRNA targeting NICDs was able to effectively inhibit the NOTCH signaling pathway and suppress its downstream targets in the EBV-positive cell line C666-1. The effects of NOTCH signaling inhibition on the transformed phenotypes of NPC cells was also determined.

141 (RegID: 1474; 1475)

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THE EPSTEIN-BARR VIRUS ONCOGENE PRODUCT, LATENT MEMBRANE PROTEIN 1, INDUCES EXPRESSION OF THYMIDINE PHOSPHORYLASE IN NASOPHARYNGEAL CARCINOMA

Chia-chun Chen, Yu-Sun Chang

Posterabstract:

Thymidine phosphorylase (TP), which was firstly identified as an enzyme involved in reversible conversion of thymidine to thymine and 2-deoxy-D-ribose-1-phosphate, also acts as an angiogenic and anti-apoptotic factor contributing to tumor progression. Elevated TP expression is often detected in various solid tumors, and considered as a tumor biomarker linked to poor prognosis. These phenomena have also been verified in nasopharyngeal carcinoma (NPC) recently (Chen et al., 2008). However, the molecular mechanisms underlying TP overexpression in tumors remain unclear. My present studies demonstrated that both mRNA and protein levels of TP could be induced by an oncogenic protein, latent membrane protein 1 (LMP1) encoded by EBV, which is closely associated with NPC. Transient transfection studies indicated that both CTAR1 and CTAR2 domains of LMP1 C-terminus could mediate TP induction based on the data using a series of LMP1 and LMP1 mutant constructs. Among specific inhibitors for LMP1-mediated signaling pathways, the dose-dependent inhibition of BAY11-7082 indicated that the nuclear factor (NF)-κB, which is activated through both CTAR domains, was involved in TP regulation. Furthermore, the ecotopically expressed p65 also upregulated the TP expression, and the constitutively active form of IκB could abolish the LMP1-mediated TP induction. All these data emphasized that the NF-κB signaling is important for TP regulation in nasopharyngeal carcinoma.

142 (RegID: 1522; 1523)

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THE RECEPTOR TYROSINE KINASE, DDR1, IS AN LMP1 TARGET GENE THAT IS ABERRANTLY EXPRESSED IN HODGKIN'S LYMPHOMA

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Posterabstract:

Although most patients with Hodgkin's lymphoma (HL) are cured, chemotherapy causes significant secondary complications in long-term survivors and new approaches to the treatment of this disease are required. HL is characterised by the constitutive activation of several signaling pathways, including STAT5 and NF-κB. The mechanisms leading to the activation of these signalling pathways are still to be elucidated, but might include the aberrant expression of receptor tyrosine kinases (RTKs). Many RTKs are targets of new generation small molecule RTK inhibitors, several of which are already in clinical use. We have focused on the regulation of RTK expression and activity by the Epstein-Barr virus (EBV)-encoded proteins.

We have shown that the RTK, discoidin domain receptor 1 (DDR1), which has been shown to be important for the activation of several cellular signalling pathways, including STAT5 and NF-κB, is over-expressed by the malignant cells of primary HL. Furthermore, all B-cell derived HL cell lines and Burkitt lymphoma cell lines examined demonstrated high-level expression of DDR1. Our studies have revealed that expression of the EBV oncoprotein, latent membrane protein 1 (LMP1), is sufficient to induce high-level expression of DDR1 in germinal centre B cells, the presumed progenitors of HL.

We are currently investigating the consequences of aberrant DDR1 activation on various signaling pathways. As DDR1 has already been described as an important activator of several molecules known to be associated with HL, the activation of DDR1 by LMP1 could represent an important pathogenic event in the development of virus-associated HL. Furthermore, because DDR1 has recently been described as an additional major target of the RTK inhibitors, dasatinib and nilotinib, we are also investigating if these drugs could be used in the future treatment of HL.

143 (RegID: 1707; 1721)

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THE EPSTEIN-BARR VIRUS (EBV)-ENCODED LATENT MEMBRANE PROTEIN-1 (LMP1) INDUCES A STATE OF KERATINOCYTE "ACTIVATION" BY DEREGULATING INTEGRIN RECEPTOR SIGNALLING

M.A. Morris, L. Laverick, J.R. Arrand, L.S. Young and C.W. Dawson

Posterabstract:

The EBV-encoded oncoprotein, LMP1, induces profound effects on the growth and differentiation of epithelial cells. LMP1 expression in the human epidermal keratinocyte cell line, SCC12F, is associated with altered cell morphology and the acquisition of a more motile phenotype. Microarray gene expression profiling reveals that LMP1 induces the expression of classes of genes that are characteristically upregulated in hyperkeratotic or "wounded" keratinocytes, suggesting that LMP1 induces a state of keratinocyte "activation". LMP1 induces the expression of interleukin (IL)-1, activin A and other TGFb family members, all of which participate in keratinocyte wound healing by promoting motility. In organotypic raft culture, LMP1 expressing cells failed to stratify and formed poorly organised structures which displayed impaired terminal differentiation. Unlike control cells, where b1 and b4 integrin expression was confined to basal cells, expression of these integrins was evenly distributed in all layers of the LMP1 raft structure. In monolayer culture, LMP1 expressing SCC12F cells show increased levels of "active" b1 integrins and display increased rates of attachment and spreading onto fibronectin. Indeed, LMP1 expressing cells themselves induce the expression and deposition of fibronectin into culture medium, indicating a greater propensity towards migration. LMP1 expressing cells show increased kinetics of focal adhesion assembly and turnover, as assessed by immunofluorescence staining for talin, vinculin, phospho-paxillin and phospho-FAK. In addition, LMP1 expressing cells migrate with faster kinetics than control cells in transwell migration assays. When cultured in suspension, LMP1 expressing cells fail to downregulate cell surface integrins, indicated by sustained phosphorylation of FAK, and maintain elevated levels of ERK-MAPK, rendering them more resistant to suspension-induced apoptosis or "anoikis". The use of selective inhibitors reveals that LMP1's ability to promote cell attachment and cell motility is mediated by maintaining integrin signalling and sustained activation of the ERK-MAPK pathway.

144 (RegID: 1712; 1713)

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THE DEUBIQUITINATING ENZYME A20 NEGATIVELY REGULATES LMP1 ACTIVATION OF IRF7

Shunbin Ning, Julia Shackelford, Joseph S. Pagano

Posterabstract:

The deubiquitinating enzyme (DUB) A20 is an anti-apoptotic protein which is induced by the EBV oncoprotein LMP1, but its role in EBV oncogenesis has not been investigated. A20 is a member of the ovarian tumor (OTU) DUB family, and its DUB activity is required for termination of TLR signaling leading to NF-κB activation, and for blockage of TNF-induced cytotoxicity and apoptosis. IRF7 has oncogenic properties, and we have recently shown that LMP1 activates IRF7 through K63-linked ubiquitination which requires RIP and TRAF6 (Huye/Ning et al. Mol. Cell. Biol, 2007; Ning et al. Mol. Cell. Biol., 2008).

In this study, we show that A20 negatively regulates IRF7 transcriptional activity induced by LMP1. Deletion or mutation of A20 C-terminal Zinc finger motifs had no effect on the inhibition of IRF7 activity, whereas deletion of the N-terminal OTU domain ablated the ability of A20 to inhibit IRF7. Correspondingly, A20 N-terminus but not C-terminus interacts physically with IRF7. Moreover, A20 interacts with IRF7 endogenously in EBV latently infected type 3 cells in which expression of both A20 and IRF7 are constitutively induced by the high level of endogenous LMP1. Knockdown of endogenous A20 in Raji cells by expression of A20 shRNA vectors increases endogenous IRF7 activity and ubiquitination. In vitro deubiquitination assay results show that IRF7 is a substrate for the DUB A20 which removes K63-linked polyubiquitin chains from IRF7. Thus, A20 acts as a DUB to negatively regulate LMP1-stimulated IRF7 activity, and may participate in regulation of LMP1 oncogenic mechanisms at least through regulation of IRF7 activity.

145 (RegID: 1730)
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EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 INDUCES EPIDERMAL GROWTH FACTOR RECEPTOR THROUGH DISTINCT NF-κB PATHWAY

Che-Pei Pat Kung and Nancy Raab-Traub

Posterabstract:

Epstein Barr virus (EBV) latent membrane protein 1 (LMP1) activates multiple forms of NF-κB through two domains in its carboxy terminus, CTAR1 and CTAR2. The canonical pathway is regulated by IkBα to produce the p50/p65 heterodimers and is activated by both domains. The non-canonical pathway which produces p52/relB heterodimers and is regulated by IKKα is activated solely by CTAR1. However, the roles of other NF-κB members, such as p50 and Bcl-3, in LMP1-mediated signaling pathways are not well understood. We have previously shown that CTAR1 uniquely induces expression of the epidermal growth factor receptor (EGFR), and p50/Bcl-3 complexes can be detected by chromatin immunoprecipitation（ChIP）analysis on the EGFR promoter in CTAR1-expressing epithelial cells and NPC cells. We show here that C33A cells expressing CTAR1 but not CTAR2 had abundant nuclear p50 and increased levels of Bcl-3. Bcl-3 was increased mainly through transcriptional induction rather than stabilization, indicated by RT-PCR and cycloheximide/MG132 analyses. Interestingly, LMP1 CTAR1 but not CTAR2 uniquely increased levels of phosphorylated STAT3, which is known to regulate Bcl-3 transcriptionally. Moreover, chemical inhibition of STAT3 significantly decreased Bcl-3 and EGFR. In CTAR1 cells, increased levels of activated STAT3 were detected by ChIP on STAT-binding sites located within both the promoter and second intron of Bcl-3. The roles of the different IKKs in EGFR induction were also assessed using mouse embryonic fibroblasts (MEFs). Preliminary results indicate that NIK, and to lesser level IKKα and IKKβ, are required for LMP1-mediated EGFR induction in MEFs. These data suggest that LMP1 activates distinct forms of NF-kB to regulate different cellular genes. In addition to the canonical and non-canonical pathways, LMP1-CTAR1 induces EGFR by constitutively activating STAT3 and increasing Bcl-3 and this particular pathway is at least partially mediated through NIK.

146 (RegID: 1737)

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EPSTEIN-BARR VIRUS LMP2A ACCELERATES MYC-INDUCED LYMPHOMAGENESIS

Rebecca Bultema, Michelle Swanson-Mungerson, and Richard Longnecker

Posterabstract:

Epstein-Barr virus (EBV) was originally identified in tumors of Burkitt's lymphoma (BL) in 1964. However, the contribution of EBV to BL is undefined. EBV encodes for multiple proteins in latent B cells that affect B cell survival and activation. One such protein, latent membrane protein 2A (LMP2A) protects B cells from numerous pro-apoptotic stimuli. Therefore, we hypothesized that LMP2A may protect B cells from apoptosis induced by aberrant Myc expression that precedes and dominates BL. To test this hypothesis, we crossed LMP2A-transgenic mice in which all B cells express LMP2A to a mouse model of BL (Myc-Tg mice). We identified that B cells from pre-tumor LMP2A/Myc-Tg mice demonstrate a significant decrease in apoptosis and an increase in the number of cycling cells when compared to Myc-Tg mice. Furthermore, pre-tumor B cells in LMP2A/Myc-Tg mice express higher levels of Myc, and Bcl-XL when compared to pre-tumor B cells from Myc-Tg mice. We hypothesized that the increase in Bcl-XL expression may accelerate the onset of tumor development in LMP2A/Myc-Tg mice. Accordingly, LMP2A/Myc-Tg mice demonstrate a significantly earlier onset of tumor development in comparison to Myc-Tg mice. These results support a hypothesis that EBV LMP2A may promote the onset of BL by protecting cells that would normally apoptose after the c-myc translocation until secondary mutations are incurred.

147 (RegID: 1740)
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EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 2 INCREASES β-CATENIN LEVELS IN EBV TYPE III LATENCY THROUGH UP-REGULATION OF UBIQUITIN C-TERMINAL HYDROLASE L1

Anjali Bheda, Julia Shackelford, Joseph. S. Pagano

Posterabstract:
Deubiquitinating enzymes (DUBs) have been increasingly implicated in regulation of cellular processes, but a functional role for Ubiquitin C-terminal Hydrolases (UCHs), which had been largely relegated to processing of small ubiquitinated peptides, remains unexplored. One member of the UCH family, UCH L1, is unexpectedly expressed in a number of malignancies suggesting that this DUB might be involved in an oncogenic process, and UCH L1 activity has been detected in EBV-infected cell lines. We have previously demonstrated that EBV Type III products activate oncogenic beta-catenin signaling in B-cells. We now identify beta-catenin as a direct target of UCH L1 deubiquitinating activity. UCH L1 deubiquitinates and stabilizes beta-catenin and up-regulates beta-catenin/TCF transcriptional function via the Lef1/TCF4 binding sites on the UCH L1 promoter. We have also implicated EBNA2 in activation of beta-catenin, and now we demonstrate that EBNA2 is involved as a transcriptional co-factor in activation of the UCH L1 promoter through binding of Pu.1 and RBP-Jk transcription factors. Our data indicate a positive feedback loop between UCH L1 and beta-catenin, where they regulate each other. Therefore, we speculate that EBNA2-mediated UCH L1 expression may be a mechanism whereby EBV maintains Type III latency associated with B-cell lymphomas.

148 (RegID: 1811)

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EPSTEIN - BARR VIRUS NUCLEAR ANTIGEN 3C CRITICALLY MODULATES UBIQUITIN-PROTEASOME PATHWAY TO REGULATE P53-MDM2 STATUS IN EBV INFECTED CELLS

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Epstein-Barr virus (EBV) stimulates the proliferation of latently infected B-cells and promotes lymphoid malignancies in humans. EBNA3C, one of the crucial latent antigens for primary B-cell transformation, is known to dysregulate ubiquitin-proteasome machinery to destabilize several vital cell-cycle regulators - Rb and p27 proteins. Interestingly, in this study we describe results which show that EBNA3C can efficiently inhibit ubiquitination/degradation of the important cellular oncoprotein, Mdm2, which is known to be overexpressed in several human cancers, is a specific ubiquitin ligase that promotes p53 ubiquitination and degradation in unstressed cells, to suppress its anti-proliferative effects. In addition, we also demonstrate that the N-terminus of EBNA3C forms a stable ternary complex with Mdm2-p53 and enhances the intrinsic ubiquitin ligase activity of Mdm2 towards p53 to facilitate its ubiquitination and degradation. Thus, manipulation of these cellular oncoproteins, p53 and Mdm2 by EBNA3C potentially provides a favorable environment for progression of EBV-infected malignant cells.

149 (RegID: 1874; 1875)
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EBNA3C TRANSCRIPTIONAL REGULATION THROUGH RBP-JKAPPA IS CRITICAL FOR GROWTH MAINTENANCE OF LYMPHOBLASTOID CELLS

Seiji Maruo 1, Yi Wu 1, Teru Kanda 1, Elliott Kieff 2, and Kenzo Takada 1, 1 Institute for Genetic Medicine, Hokkaido University, 2 Brigham and Women's Hospital, Harvard Medical School

Posterabstract:
Although EBNA3C is essential for EBV-induced primary B cell outgrowth into lymphoblastoid cell lines (LCLs), the precise mechanisms by which EBNA3C contributes to LCL growth are still unclear. We evaluated the ability of EBNA3C mutants to support the maintenance of LCL growth and found that an EBNA3C mutant lacking aa 180 to 231, which was defective in RBP-Jkappa association, was unable to support LCL growth maintenance. Moreover, analyses using a series of EBNA3C point mutants in which alanine substitution mutations were introduced within aa 180 to 231 revealed that the ability of EBNA3C point mutant to regulate transcription through RBP-Jkappa was well correlated with its ability to sustain LCL growth maintenance. The data indicates that EBNA3C regulation of transcription through RBP-Jkappa is critical for maintaining LCL growth.
We also examined the specific roles of p16INK4A and p14ARF in the growth arrest of LCLs induced by EBNA3C inactivation. Inactivation of EBNA3C in LCLs resulted in transcriptional induction of p16INK4A and p14ARF, which was accompanied by growth arrest of the LCLs. Transfection of plasmids containing shRNAs targeting p16INK4A and p14ARF rescued the LCLs from the growth arrest. Thus, p16INK4A and p14ARF repression may be a critical EBNA3C function.

150 (RegID: 1924; 1925)

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TGF-β1-INDUCED EBV REACTIVATION REQUIRES PI3-K/AKT FOR ASSOCIATION OF THE CO-ACTIVATOR CBP WITH SMAD3. LATE LYTIC PROGRAM LEADS TO A TWO STEPS DOWN-REGULATION OF EXPRESSION OF THE PROAPOPTOTIC BIMEL PROTEIN

L. OUSSAIEF, A. HIPPOCRATE, V. RAMIREZ C. CLYBOUW, A. VAZQUEZ, A. RAMPANOU, I. JOAB

Posterabstract:

Epstein-Barr virus (EBV) is associated with the development of carcinomas and lymphomas. We previously showed that Transforming Growth Factor Beta 1 (TGF-β1) switches the virus for latency into the lytic cycle by triggering expression of ZEBRA. We explored the cascade of signalling events required for TGF-β1-mediated-EBV reactivation. We showed that activation of NF-κB followed by phosphorylation of ErK 1/2 were responsive for phosphorylation of Akt. ZEBRA expression was dependent on PI3-K/Akt signalling pathway, since phosphoAkt enables Smad3, a mediator of TGF-β1 signalling, to be acetylated by direct interaction with the co-activator CREB-binding protein (CBP), and then regulate TGF-β1-induced ZEBRA expression. These results provide new findings for TGF-β1-induced EBV reactivation, and identify PI3-K/Akt signalling pathway as a critical regulator of Smad3-CBP association and Smad3 acetylation in an EBV- latently infected Burkitt's lymphoma cell lines.

Investigation of the modulation of apoptotic signals during lytic cycle was performed: a two steps proteasome-dependent down-regulation of expression of the proapoptotic protein BimEL occur during EBV reactivation. The first drop was ERK 1/2 dependent and might be EBV-independent and a second dramatic drop of the BimEL level was observed during the late phase of the lytic cycle and was dependent of EBV-late gene expression. Semi-quantitative RT-PCR analysis showed that the levels of mRNA corresponding to BimEL were similar in both uninduced and induced EBV positive BL cell lines, suggesting that the lower levels of BimEL protein observed upon EBV reactivation were not due to a decrease of the steady state in RNA level by likely to a post-transcriptional mechanism.

Our findings demonstrated that anti-apoptotic signals are upregulated during EBV reactivation and that the lytic cycle contributes directly or indirectly to down-regulation of BimEL and then could add to protection by EBV lytic cycle against apoptosis.

151 (RegID: 1926)
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EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 3C REPRESSES

Fuming Yi, Masanao Murakami, Pankaj Kumar, Abhik Saha, Jason S. Knight, Qiliang Cai, Tathagat Choudhuri,Erle S. Robertson

Posterabstract:

The p53 tumor suppressor protein plays a central role in the process of apoptosis and the cellular defense against abnormal cell proliferation such as tumor development. It is widely known that the p53 mutation or lack of p53 expression causes tumors, and this is important in the development of a lot of tumors and cancers. Moreover, it has been known that some tumor viral proteins which SV40 large T antigen, adenovirus E1A and HPV E7 interact with p53 and disrupt its function. Here, we show new partner of p53 which is identified one of the tumor viruses antigen; Epstein-Barr virus (EBV) nuclear antigen (EBNA) 3C. EBNA3C interacts directly with p53 in vitro and in vivo. EBNA3C is one of the essential proteins for the EBV induced B cell immortalization. This interaction between EBNA3C and p53 was mapped onto the EBNA3C 130-190 amino acid residues which we also identified as a binding site to the SCFSkp2 complex previously. We demonstrated that p53 transcriptional activity was decreased dose dependent manner of EBNA3C. Moreover, EBNA3C expressing SAOS-2 cell line which is EBNA3C transfected p53-null cell line, showed repression of the apoptosis ratio by wild type p53 expression. Furthermore, we demonstrate that EBNA3C might regulate p53 by inhibiting its DNA-binding ability. Thus, EBNA3C may play an important role in the cell cycle regulation through the association with p53 protein.

NOTES:

Poster sessions

Session 5: Immune Mechanisms

152 (RegID: 1205)
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LMP2A AS A TARGET FOR T CELL RECOGNITION IN EBV-ASSOCIATED T AND NK CELL TUMOURS

Christopher P. Fox, Heather M. Long, Claire-Shannon Lowe, Norio Shimizu, Alan Rickinson and Martin Rowe

Posterabstract:
Expression of viral antigens opens the possibility of targeting EBV-positive tumours with cells specific for these antigens. As a preliminary, however, one has to know whether the tumour cells have intact antigen processing pathways, and what range of EBV antigens they express. EBV is associated with particular tumours of T and NK cell origin. Our analysis of cell lines derived from four such tumours, SNT8 and SNT16 of T cell origin and SNK6 and SNK10 of NK cell origin, has demonstrated expression of HLA class I and the TAP transporter complex proteins, indicating that the antigen processing pathway for CD8+ T cells was intact. Regarding EBV antigen expression, EBNA1 and LMP1 were detectable by immunoblotting in all lines, whereas, using a monoclonal antibody capable of detecting the protein in EBV-transformed lymphoblastoid cell lines, LMP2A was not. However, consistent with previous reports, LMP2A mRNA transcripts were detected in all lines. To ask whether this level of expression was sufficient to enable recognition by LMP2-specific T cell clones, we used three CD8+ T cell clones recognizing LMP2 epitopes (FLYALALLLL/A2, CLGGLLTMV/A2, TYGPVFMCL/A24) restricted through the HLA-A2 or HLA-A24 alleles expressed by the SNT16 line. We observed specific recognition and lysis of the tumour cell line by these clones in interferon–release and cytotoxicity assays, respectively. These results suggest that, even where the expression of LMP2 is low in EBV-positive NK/T tumours, the levels may still be sufficient to serve as a target for appropriately directed CD8+ T cell therapy.

153 (RegID: 1242)
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STRONG CORRELATIONS OF ANTI-VIRAL CAPSID ANTIGEN ANTIBODY LEVELS IN FIRST-DEGREE RELATIVES FROM FAMILIES WITH EPSTEIN BARR VIRUS-RELATED LYMPHOMAS

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Posterabstract:
Background: Markers of Epstein-Barr virus (EBV) infection include anti-VCA IgG. High anti-VCA titers are associated with EBV-related lymphoproliferations, such as Burkitt (BL) and Hodgkin lymphomas (HL).

Methods: Intrafamilial correlations of anti-VCA IgG were studied in three settings: 127 families recruited through HL cases in France (A), 31 families recruited through BL cases in Uganda (B), and 74 large families from a general population from Cameroon (C). Titers were determined by ELISA (A and C) and immunofluorescence analysis (B).

Results: In samples A and B, the anti-VCA IgG titers of relatives of HL and BL patients increased significantly (P=0.01 and =0.0009, respectively) with those of the index case. In all three samples, anti-VCA IgG titers were significantly correlated (P=2 x 10-8 for A, 2 x 10-3 for B, and 10-10 for C) between genetically related individuals (father-offspring, mother-offspring and sib-sib), but not between spouses. Similar results were obtained for sample A after adjustment for total IgG levels. In all cases, the pattern of correlations was consistent with a polygenic model, heritability ranging from 32% to 48%.

Conclusion: These results provide evidence for the genetic control of anti-VCA IgG titers and pave the way for the identification of the locus involved.

154 (RegID: 1245)
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LMP1 TRAFFICKING TO EXOSOMES THROUGH THE ENDOCYTIC PATHWAY

Pegtel DM, Eijndhoven ME, Hopmans E, Neefjes JJ, Middeldorp JM

Posterabstract:

Exosomes are nano-sized vesicles and function as intercellular signaling devices that originate from intraluminal vesicles (ILV's) of multi-vesicular multivesicular-bodies (MVBs). In MVBs of antigen presenting cells (APCs), ILV's facilitate class II peptide loading. APC derived exosomes also harbor co-stimulatory molecules and perhaps not surprisingly, these vesicles are potent inducers of T cell activation. Several pathogens have evolved to hijack the highly specialized MVB antigen processing pathway and negatively influence immune recognition.

In EBV-infected lymphoblastoid B cell-lines, only a small fraction of the endogenous latent membrane protein 1 (LMP1) is present on the plasmamembrane, while the bulk of LMP1 resides in poorly defined intercellular compartments. We show that endogenous LMP1 is co-expressed with the tetraspannin CD63 a marker for early and late endosomes. Furthermore, when cells are treated pharmacologically with wortmannin and/or chloroquine, we detect intracellular LMP1 and HLA-DR in distinct microdomains of presumably MVBs. A recent study proposes that exosomes may originate from (lipid) micro-domains in the limiting membrane of MVBs1. Interestingly, both HLA-DR and LMP1 proteins are known to aggregate in "lipid raft like" domains of the plasmamembrane. We confirmed by electron microscopic immunogold labeling that LMP1 protein is present in HLA-DR positive exosomes. In addition, lysates of exosomes captured by anti-HLA-DR coupled dynal-beads contain significant amounts of LMP1 protein. These results show for the first time that endogenous LMP1 is secreted via exosomes by trafficking to intercellular compartments, specialized in antigen loading. Due to the known immunosuppressive properties of LMP1, we propose that LMP1 may have co-opted the endosomal sorting pathway to manipulate antigen presentation and/or exosome function.

155 (RegID: 1251; 1253)
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MONITORING OF EPSTEIN-BARR VIRUS LOAD AND KILLER T CELLS IN RENAL TRANSPLANT RECIPIENTS.

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Posterabstract:
Aim: The aim of this study is to establish a monitoring method to prevent Epstein-Barr virus (EBV) -associated symptoms including post-transplant lymphoproliferative disorder (PTLD) that occur after pediatric renal transplantation. Subjects and Methods: Circulating EBV loads were quantified by real-time PCR every 1 to 3 months after grafting in 22 pediatric recipients (13 EBV-seronegative [R(-)] and 9 EBV-seropositive [R(+)] recipients before grafting). The peripheral blood cell populations of non-specific activated killer cells (CD8+HLA-DR+ phenotype) in 13 R(-) recipients and EBV-specific cytotoxic T cells (CTLs) reactive with a tetramer expressing HLA-A24-restricted EBV-specific antigens in 8 of 13 R (-) recipients were determined by flow cytometry. Results: EBV-associated symptoms including PTLD (two cases) were found in 4 R(-) and none of R(+) recipients. The maximum of EBV load in R (-) group was significantly higher that in R(+) group. In R (-) recipients, 4 symptomatic cases had significantly more EBV genome than asymptomatic cases. EBV-specific CTLs were detected in 6 of the 8 R (-) recipients, but these CTLs could not be detected in one of the two cases at onset of PTLD. The percentage of CD8+HLA-DR+ cells was significantly higher in asymptomatic recipients than in recipients with EBV-associated symptoms whose EBV loads were over 400 copies /μg DNA. Conclusion: Monitoring of killer T cells and EBV loads may allow assessment of the risk of EBV-associated symptoms, and high EBV loads and low EBV-specific and/or non-specific CTL responses may be predictive for development of EBV-associated symptoms such as PTLD.

156 (RegID: 1260)
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PRIMING OF PROTECTIVE AND TUMOR SPECIFIC T CELL RESPONSES IN MICE WITH HUMAN IMMUNE SYSTEM COMPONENTS

Till Strowig, Cagan Gurer, Alexander Ploss, Yi-Fang Liu, Frida Arrey, Junji Sashihara, Charles Rice, James W. Young, Amy Chadburn, Jeffrey I. Cohen, and Christian Münz

Posterabstract:

Several pathogens demonstrate an exquisite selectivity in their tropism for humans. In order to study the protective correlates of immune control for these pathogens in vivo and also to evaluate promising vaccine candidates, a small animal model that could be challenged with these pathogens would be desirable. We demonstrate here that infection with the oncogenic and persistent Epstein Barr virus (EBV) can be controlled by primary T cell responses in mice with reconstituted human immune system components. Even so EBV was able to establish viral latency, massive expansion of EBV specific T cells controlled the infection. These T cell responses were HLA restricted and partially specific for EBV derived peptides. They mediated the protective effector functions of IFN-g production and cytotoxicity. Depletion of T cells resulted in elevated viral loads and emergence of EBV associated lymphoproliferative disease. We suggest that this in vivo model of protective T cell response priming recapitulates symptomatic primary EBV infection, and should be evaluated for vaccine testing against EBV and other pathogens, relevant to human health.

157 (RegID:1301; 1302、1303)

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IN VITRO STIMULATION WITH AUTOLOGOUS EBV-TRANSFORMED B CELLS REACTIVATES CD4+ T CELL RESPONSES THAT RECOGNISE HUMAN B LYMPHOMA-ASSOCIATED CELLULAR ANTIGENS

Long HM, Zuo J, Leese AM, Gudgeon NH, Jia H and Rickinson AB
CR-UK Institute for Cancer Studies, University of Birmingham, Birmingham, UK

Posterabstract：

Epstein-Barr virus (EBV)-specific T cell preparations, generated by stimulating immune donor lymphocytes with the autologous EBV-transformed B lymphoblastoid cell line (LCL) in vitro, are being used to target EBV-positive malignancies. Whilst these preparations are enriched for EBV antigen-specific CD8+ T cells, most contain a small CD4+ T cell population whose specificity is unknown.

As one might anticipate of an EBV antigen-specific response, CD4+ T cells generated in this way recognise EBV-transformed LCLs and not mitogen-activated B or T lymphoblasts or dendritic cells, and at the clonal level are classically restricted to one particular HLA-DR, -DQ or -DP allele. However, three pieces of evidence show that these LCL-stimulated CD4 effectors are not EBV-specific. Firstly they do not map to any known EBV antigen or epitope in recombinant vaccinia or peptide sensitisation assays. Secondly they can be raised from EBV-naïve as well as from EBV-immune individuals. Thirdly, they are capable of recognising a broad range of human B-lymphoma-derived cell lines irrespective of EBV genome status, providing those lines express the relevant HLA class II allele. Importantly, while all of the CD4+ clones produce IFNγ, many are also cytotoxic and can control the outgrowth of their target cells in co-cultivation assays.

We infer that such CD4+ T cells are capable of recognising cellular antigens whose expression is specifically up-regulated in normal B cells by EBV-infection. These same cellular antigens also appear to be up-regulated in human B lymphoma cells, where their presentation via the HLA class II pathway renders these cells open to recognition by LCL-stimulated effectors. The identity of these antigens and their means of presentation are under investigation.

158 (RegID: 1301; 1302; 1303）

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T CELL RESPONSES AGAINST BHRF1, RECENTLY RE-DEFINED AS AN EBV LATENT ANTIGEN

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Posterabstract:

Cellular immune responses to EBV are important for the control of infection in immunocompetent individuals, and have potential therapeutic use in patients with EBV-associated malignancies. However, most of the EBV-associated malignancies show restricted forms of viral antigen expression, and thus limited availability of antigenic targets for the T cell response. Recent work in our laboratory has demonstrated that the viral Bcl-2 homolog, BHRF1, a protein usually associated with the virus lytic cycle, is in fact constitutively expressed in B lymphoblastoid cell lines transformed with a BZLF1-knockout virus and therefore incapable of lytic cycle entry. Furthermore, BHRF1 is also expressed as a latent protein in that subset of Burkitt lymphomas using the Wp rather than the Qp promoter, thereby extending the range of potential T cell target antigens beyond EBNA1.

Here we have conducted a systematic study of BHRF1 as a target for both CD8+ and CD4+ T cell responses. Peripheral blood mononuclear cells from 17 healthy virus-immune donors were screened ex vivo by interferon gamma ELISpot assay against a panel of overlapping 15mer peptides covering the primary sequence of BHRF1. CD8+ memory T cell responses were detectable in 3 donors against 3 individual peptides, while eight donors had detectable CD4+ T cell responses to one or more of 7 peptides. BHRF1-specific clones established from the above donors were able to recognise both wild-type and BZLF1-ko LCLs in assays of IFNγ release. Furthermore, we were able to detect recognition of autologous B cells within 4 days of infection with a recombinant BZLF1-knockout virus, demonstrating that BHRF1 is expressed, processed and presented to T cells at an early stage of EBV-induced B cell transformation.

159 (RegID: 1355; 1383)

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HIGH PERFORIN EXPRESSION ON T CELLS: A PROGNOSTIC MARKER FOR SEVERITY OF HERPESVIRUS REACTIVATION EARLY AFTER STEM CELL TRANSPLANTATION

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(4) Department of Hematology, Erasmus Medical Center, Rotterdam, the Netherlands

Posterabstract:

Viral reactivations are a major complication after stem cell transplantation (SCT). Due to the immunosuppressed state of the transplant recipient, reactivation with Epstein-Barr virus (EBV) cannot be adequately controlled by the immune system and can lead to uncontrolled lymphoproliferation and subsequent post-transplant lymphoproliferative disease (PTLD). Cytomegalovirus (CMV) infection or reactivation is also a major cause of morbidity and mortality following SCT. Since EBV and CMV reactivations frequently coincide in SCT recipients we classified patients as with or without herpesvirus reactivation based upon their peak viral load of either virus. Detection of reactivation and subsequent pre-emptive therapy relies on frequent viral load detection after stem cell transplantation. Additional virus specific T-cell reconstitution data can increase the predictive value for viral complications following transplantation. Here we studied perforin expression in CD8+ T cells, used as a measure for cytotoxic T-cell capacity, in relation to occurrence of viral reactivation. In a prospective study we followed 40 individuals during the first 3 month post transplantation and measured viral load levels in combination with intracellular perforin expression in CD8+ T cells. Median intracellular perforin levels in CD8+ T cells throughout 3 months post SCT are higher in patients with herpesvirus reactivations (4,9% of CD8+ T cells versus 2,3% of CD8+ T cells, p=0,001). Perforin levels increase with reactivation severity, patients with low viral loads had median perforin levels of 3,9% compared to 10,7% in patients with viral loads exceeding 1000 copies/ml. Patients with viral reactivations exceeding 1000 copies/ml in plasma reached high percentages of intracellular perforin in CD8+ T cells much more often and much faster than patients with low (viral load below 1000 copies/ml) or without reactivation. In all cases perforin expression preceded high viral loads making perforin an easy to use prognostic marker to identify patients at risk of severe viral reactivations.

160 (RegID: 1396)
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EVASION FROM CYTOTOXIC T CELL IMMUNITY DURING PRODUCTIVE EBV INFECTION: THE CONCERTED ACTION OF MULTIPLE GENES

Maaike Ressing1, Daniëlle Horst1, Andrew Hislop2, Nathan Croft2, Jianmin Zuo2, Daphne van Leeuwen1, Elisabeth Kremmer3, Bryan Griffin1, Martin Rowe2, Alan Rickinson2, Emmanuel Wiertz1,
1 Dept. Medical Microbiology, Leiden University Medical Center, The Netherlands, 2 Div. Cancer Studies, University of Birmingham, United Kingdom, and 3 Inst. of Molecular Immunology, GSF, Germany

Posterabstract:
EBV persists for life in infected individuals despite the presence of antiviral immunity. Especially cytotoxic T-lymphocytes are extremely effective in detecting viral infections, through the sampling of MHC class I-peptide complexes displayed at the cell surface. The fact that the immune system nevertheless fails to eliminate cells harbouring replicating EBV indicates that this virus must have evolved highly effective strategies to elude the host immune response.
The establishment of an in vitro system for synchronized EBV replication facilitated the isolation of pure populations of B cells in the productive phase of infection. A strong downregulation of MHC class I molecules was observed at the surface of productively infected cells. In addition, the transporter associated with antigen processing (TAP) was inhibited, resulting in reduced transport of peptides into the ER for presentation by MHC class I molecules. These observations led us to search for immune evasion functions encoded by EBV lytic cycle genes.
We report the concerted action of EBV lytic phase proteins, preventing the expression of MHC class I molecules at the cell surface. BNLF2a inhibits TAP-mediated peptide transport by blocking both ATP and peptide binding to TAP. In addition, synthesis of cellular proteins, including MHC class I molecules, is blocked by the viral exonuclease, BGLF5. This protein also acts as a virus host shutoff protein, inducing mRNA degradation. TAP expression is not affected by BGLF5, which may be related to the fact that TAP has a very long half life.
Thus, BGLF5 and BNLF2a act synergistically, the former blocking synthesis of new MHC class I molecules and the latter preventing peptide-loading of existing MHC class I molecules. The concerted action of these lytic phase proteins creates a time window for the generation of viral progeny in the face of existing T cell immunity.

161 (RegID: 1454)
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CO-CULTURE WITH MONOCYTES ENHANCED THE EXPRESSION OF LMP-1 IN CELL LINES DERIVED FROM EBV POSITIVE NK MALIGNANCIES.

Hideyuki Ishii, Miki Takahara, Yasuaki Harabuchi, Eva Klein

Posterabstract：
Background: Nasal NK/T-cell lymphoma originates from NK or γδ T cells. The tissue is granulomatous and necrotic. The EBV positive lymphoma cells have the Type II pattern. Maintenance of cell lines derived from this tumor require IL-2. Earlier we found that expression of LMP-1 in vitro was enhanced by cytokines. These result suggested that external stimuli induced LMP-1 expression in vivo as well. Since several types of inflammatory cells surround the tumor cells in the lymphoma, we tried to identify the source of the cytokines. As a first approach, we initiated mixed cultures containing blood derived monocytes and EBV positive NK lines.

Methods: We used SNK6 that was established from a nasal NK cell lymphoma and KAI3, originated from a patient with chronic active EBV infection. Monocytes and granulocytes were separated from buffy coat of healthy donors. SNK6 or KAI3 cells were cultured with or without the inflammatory cells for 48 hrs. Mixed cultures were initiated also in transwell chambers. LMP-1 expression was monitored in immunoblot and in flow cytometry. Proliferation of the cells was estimated by visual cell count. Moreover, the presence of monocytes was investigated in biopsy samples by detection of the marker CD14.

Results: In absence of added cytokines, LMP-1 expression of SNK6 and KAI3 cells was enhanced in the mixed cultures containing monocytes but not in those containing granulocytes. Cell contacts were necessary; these parameters were not detected in the transwell cultures. In immunohistochemical analysis, using the CD14 marker revealed the existence of monocytes in the tumor tissue.

Conclusions: These results suggest that direct contact with monocytes contribute to the induction of the EBV encoded LMP-1 expression and to the proliferation of the malignant cells in the nasal lymphoma.

162 (RegID: 1496)

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NATIVE CONFORMATION AND WILD-TYPE SEQUENCE OF EPSTEIN-BARR VIRUS PROTEIN BARF1 ARE ESSENTIAL FOR HUMORAL IMMUNE RESPONSES TO BARF1 IN NASOPHARYNGEAL CARCINOMA PATIENTS.

EK Hoebe, SH Hutajulu, DK Paramita, AE Greijer and JM Middeldorp. VU University medical center, Dept. Pathology, Amsterdam, The Netherlands.

Posterabstract:

Nasopharyngeal Carcinoma (NPC) is highly prevalent in Indonesia and 100% associated with Epstein-Barr virus (EBV). NPC tumour cells express viral proteins EBNA1, LMP1, LMP2 and BARF1. The BARF1 protein is thought to have oncogenic and immune-modulating properties. This study analyzes the humoral immune response against BARF1 in Indonesian NPC patients.

EBV B95-8 BARF1 was expressed in E.coli and baculovirus yielding high levels of monomeric protein. B95-8 BARF1 derived peptides were made covering predicted antigenic domains. None of these BARF1 reagents showed a detectable interaction with IgG or IgA antibodies in NPC sera.

Based on BARF1 sequence analysis in over sixty Indonesian NPC isolates (3 AA-mutations) a NPC-BARF1 expressing human cell line was developed. Purified secreted “native” hexameric BARF1 protein was produced and used in ELISA, with sera of Indonesian NPC patients (n=155), Caucasian and Indonesian healthy EBV-positive volunteers (n=56) and EBV-negative individuals (n=33).

A clear but weak IgG and IgA response against BARF1 was observed in NPC patients having higher titers than regional controls ($p=0.02$ resp. $p<0,01$) Specificity of the response was confirmed with Immunoblot analysis. The Positive Predictive value of the BARF1-IgG ELISA is 100%, the Negative Predictive Value is 28%.

In conclusion, BARF1 is a protein with low immunogenicity for humoral responses and requires native conformation for antibody binding. The presence of antibodies against native BARF1 in the blood of NPC-patients supports evidence that this protein is expressed and secreted as hexamer in NPC tumours.

163 (RegID: 1647)
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PROCESSING OF EPSTEIN-BARR VIRUS-CODED NUCLEAR ANTIGENS FOR CD4+ T CELL RECOGNITION

CS Leung, TA Haigh, HM Long, AB Rickinson & GS Taylor

Posterabstract:

Epstein-Barr virus (EBV)-positive malignancies are frequently HLA class II-positive, raising interest in the possibility of their recognition by CD4+ T cells specific for endogenously expressed viral proteins. We have established CD4+ T cell clones against many epitopes in four EBV nuclear antigens (EBNAs) presented by defined HLA II alleles. The ability of these clones to directly recognise EBV-transformed lymphoblastoid cell lines (LCLs) expressing the target antigens at physiologic levels varies markedly between epitopes, in a way that is not related to the clone's functional avidity in peptide titration assays. Using clones showing strong LCL recognition and specific for epitopes in EBNA2 and EBNA3C, we found that the presentation of these epitopes to CD4+ T cells required antigen release and subsequent uptake processing by co-resident cells in the LCL culture; inhibition of a potential intra-cellular route, through macro-autophagy, actually increased rather than decreased EBNA2 and 3C epitope presentation. By contrast the processing of another CD4+ T cell target, EBNA1, appears not to involve inter-cellular antigen transfer but to occur via an intra-cellular pathway.

164 (RegID: 1684; 1686)
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DEVELOPMENT OF POLYCHROMATIC FLOW CYTOMETRY ASSAY TO STUDY POLYFUCNTIONAL EPSTEIN-BARR VIRUS (EBV)-SPECIFIC CD8+ CYTOTOXIC T-LYMPHOCYTE RESPONSES

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Posterabstract:

Aim: Development of effective virus-specific CD8+ cytotoxic T lymphocyte (CTL) responses is thought to be crucial in the long term control of Epstein-Barr virus (EBV). We aim to establish a polychromatic flow cytometry assay to study the development of polyfunctional EBV-specific CD8+ CTL responses.

Methods: Staphylococcal enterotoxin B (SEB) was used to stimulate cryopreserved peripheral blood mononuclear cells (PBMC) of healthy donors to simulate cytokine and chemokine secretion. Intracellular cytokine assay for three cytokines (IFN-γ, TNF-α and IL2) and one chemokine (MIP1-α) was developed. Cells were stained simultaneously for aqua blue dye, CD3, CD8, CD27, CD45RO and the three cytokines and one chemokine and analyzed by nine-color flow cytometry protocol on BD FACSAria. Setting of PMT voltage of each channel, compensation adjustments and titration of antibodies were performed. Biological (no SEB or peptide stimulation) and FMO (fluorescence minus one) controls served as negative controls.

Results: PMT voltage of each channel was determined by unstained PBMC. Compensation beads labeled with antibody of each color showed strong positive signals which were used for compensation adjustments. Aqua blue dye labeled the dead cells. Serial titration of each antibody against the cytokines was essential to determine the optimal concentration for high positive signal to low background. The background of the biological control was as low as 0.001% while the SEB-stimulated positive control population ranged from 1-20% in different samples. FMO control was used to guide the gating of CD45RO and CD27 expression.

Conclusion: A sensitive polychromatic flow cytometry assay for the evaluation of polyfunctional EBV-specific CTL is established. Single EBV peptide is used to stimulate cryopreserved PBMC of primary infection subjects with known EBV peptide responses.

165 (RegID: 1741)
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EBV LMP1 INDUCES THE SUMOYLATION OF INTERFERON REGULATORY FACTOR 7

Gretchen L. Bentz,1 Shunbin Ning,1 and Joseph S. Pagano1,2.

Posterabstract:
Interferon regulatory factor (IRF) 7, a transcription factor that is a key regulator of Type I interferons (IFN α/β) in innate and adaptive immune responses in viral infections, is constitutively expressed in lymphoid cells. Increased expression can be induced by various stimuli including the EBV oncoprotein LMP1, a constitutively active membrane protein that mimics the signaling induced by the TNF receptor family and is required for EBV transformation. Previous work in our laboratory demonstrated that LMP1 does activate IRF7. Activation is accomplished through the post-translational modification of IRF7 by both phosphorylation and K63-linked ubiquitination and its nuclear translocation. Therefore, we investigated other post-translational modifications of IRF7 that might be initiated by LMP1. One such modification is by small ubiquitin-like protein modifier (SUMO)-1, which regulates protein function through various mechanisms. Sumoylation of IRF1 results in its transcriptional repression, and we initially hypothesized that sumoylation of IRF7, induced by LMP1 during EBV latency during EBV latency, regulates its transcriptional activity and inhibits the induction of immune responses. We now show that IRF7 interacts with Ubc9, the only reported SUMO-conjugating enzyme, and that IRF7 is sumoylated, endogenously in EBV transformed lymphoblastoid cells and as well as when over-expressed exogenously, resulting in its increased nuclear accumulation. In addition, sumoylated IRF7 is only detected when LMP1 is co-expressed, suggesting that LMP1 induces IRF7 sumoylation. Our investigations into the function of sumoylated IRF7 revealed that sumoylation resulted in the activation of the Type I interferons, and further studies are in progress to examine the mechanism by which this occurs. Recently it has been shown that murine IRF7 is sumoylated following vesicular stomatitis virus infection. Our new data are the first to show that a specific viral protein induces the sumoylation of IRF7 and identify a potential target for regulation of anti-viral immune responses.

166 (RegID: 1751)

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EBV NUCLEAR ANTIGENS EBNA3A AND EBNA3C REUGLATED CELLULAR GENES

B Zhao, J Mar, S Maruo, K Holton, E Johannsen, J Quackenbush, E Kieff

Posterabstract:

EBV nuclear proteins EBNA3A and EBNA3C are essential for EBV mediated B lymphocyte transformation to lymphoblastoid cell lines (LCLs) in vitro. Both EBNA3A and 3C modulate the DNA binding activity of cellular protein CSL and genetic analyses have shown that CSL/EBNA3A/C binding is critical for the maintenance of continuous LCL growth. Thus EBNA3A and EBNA3C may contribute to LCL growth by acting at CSL regulated promoters. To identify EBNA3A and 3C regulated cell genes, LCLs were generated by transformation of PBMC with EBV recombinants that have EBNA3A or 3C fused in frame to a mutant estrogen receptor hormone binding domain, which is activated by 4-hydroxy tamoxifen. LCL growth and EBNA3AHT or EBNA3CHT expression were dependent on 4HT. Total cell RNA were prepared from 3 independent clones of EBNA3AHT and EBNA3CHT LCLs that were grown in the presence or absence of 4HT for 1, 2, 4 or 8 days. Changes in cell mRNAs have been identified using Affymetrix U133 plus 2 oligonucleotide gene arrays. By Linear Models for Microarray Data (LIMMA) analyses, 95 and 137 cell RNAs were differentially regulated by EBNA3A or 3C respectively during the time course with $P<0.01$. In addition, 429, 89, 70 and 453 cell RNAs were differentially regulated by EBNA3A at day 1, 2, 4 and 8 with $P<0.01$. 561, 119, 225 and 101 cell RNAs were differentially regulated by EBNA3C at day 1, 2, 4 and 8 with $P<0.01$. These cell genes are involved in a wide variety of cellular pathways such as transcription regulation, cell signalling, and survival. Currently, we are using realtime RT-PCR to confirm the changes identified by array analyses. RNAi knockdown will determine if these EBNA3A or 3C induced changed are essential for LCL growth and survival.

167 (RegID: 1770; 1771; 1772; 1953)

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EPSTEIN-BARR VIRUS BGLF4 KINASE SUPPRESSES THE INTERFERON REGULATORY FACTOR 3 SIGNALING PATHWAY

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Posterabstract:

BGLF4 protein kinase of Epstein-Barr virus (EBV) is a member of the conserved family of herpesvirus protein kinases which, to some extent, have a similar function to the cellular cell cycle dependent kinase in regulating multiple cellular and viral substrates. In a yeast two-hybrid screening assay, a splicing variant of interferon regulatory factor 3 (IRF3) was found to interact with BGLF4 protein. This interaction was defined further by co-immunoprecipitation in transfected cells and GST pull-down in vitro. We show, using reporter assays, that BGLF4 effectively suppresses the poly(I:C)-stimulated IFN promoter and IRF3 responsive element activities. Moreover, BGLF4 represses poly(I:C)-stimulated expression of endogenous IFN mRNA and phosphorylation of STAT1 at Tyr701. In searching for a possible mechanism, BGLF4 was shown not to affect dimerization, nuclear translocation, or CBP recruitment of IRF3 upon poly (I:C) treatment. Notably, BGLF4 reduces the amount of active IRF3 recruited to the IRF3 responsive element (IRE) containing the IFN promoter region in a ChIP assay. BGLF4 phosphorylates GST-IRF3 in vitro, but Ser339-Pro340 phosphorylation-dependent, Pin1-mediated down regulation is not responsible for the repression. Most importantly, we found three proline-dependent phosphorylation sites at Ser123, Ser173 and Thr180, which cluster in a region between the DNA binding and IRF association domains of IRF3, contribute additively to BGLF4 mediated repression of IRF3(5D) transactivation activity. IRF3 signaling is activated in reactivated EBV positive NA cells, and knockdown of BGLF4 further stimulates IRF3 responsive reporter activity. The data presented here thus suggest a novel mechanism by which herpes viral protein kinases suppress host innate immune responses and facilitate virus replication.

168 (RegID: 1781)

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REGULATION OF SP100A SUBNUCLEAR LOCALIZATION AND TRANSCRIPTIONAL FUNCTION BY EBNA-LP AND INTERFERON

Chisaroka W. Echendu and Paul D. Ling

Posterabstract:

Epstein-Barr Virus (EBV) efficiently immortalizes human B cells and is associated with several human malignancies. The EBV transcriptional activating protein EBNA2 and the EBNA2 coactivator EBNA-LP are important for B cell immortalization. Recent observations from our laboratory indicate that EBNA-LP coactivation function is mediated through interactions with the interferon-inducible gene (ISG) Sp100, resulting in displacement from its normal location in promyelocytic leukemia nuclear bodies (PML NBs) into the nucleoplasm. The EBNA-LP- and Interferon-mediated mechanisms that regulate Sp100 subnuclear localization and transcriptional function remain undefined. To clarify these issues, we generated a panel of Sp100 mutant proteins to ascertain whether EBNA-LP induces Sp100 displacement from PML NBs by interfering with Sp100 dimerization or through other domains. In addition, we tested EBNA-LP function in Interferon treated cells. Our results indicate that Sp100 dimerization, PML NB localization, and EBNA-LP interaction domains overlap significantly. We also show that IFN-β does not inhibit EBNA-LP coactivation function. The results suggest that EBNA-LP might play a role in EBV-evasion of IFN-mediated antiviral responses.

169 (RegID: 1784)

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DECREASED VIRUS-SPECIFIC CD8+ T CELLS IN PATIENTS WITH EPSTEIN-BARR VIRUS ASSOCIATED NASOPHARYNGEAL CARCINOMA

Mark H Fogg, Lori J Wirth, Marshall Posner, and Fred Wang

Posterabstract:

Background: The Epstein-Barr virus (EBV) Nuclear Antigen-1 (EBNA-1) is potentially a universal target for immune recognition of EBV-infected normal or malignant cells. Yet EBNA-1-specific CD8+ T cell responses have only been assessed in detail against a limited number of epitopes presented on select HLA class I alleles. We now assess CD8+ T cell responses to a panel of EBNA-1 and Latent Membrane Protein 2 peptides in an HLA characterized populations of healthy EBV immune individuals and individuals with EBV-associated nasopharyngeal carcinoma (NPC).

Methods: EBV-specific T cells were stimulated from the peripheral blood of 13 healthy donors and 17 NPC patients using autologous EBV-immortalised B cell lines. EBNA-1 and LMP2-specific activity was assessed in these T cell lines by their peptides spanning EBNA-1 or LMP2 in interferon-gamma ELISPOT assays.

Results: EBNA-1- and LMP2-specific CD8+ T cells were detected in a similar frequency of T cell lines generated from EBV-immune healthy donors. EBNA-1 epitopes were shown to be HLA class I restricted by intracellular cytokine staining and half were in not previously described HLA backgrounds, demonstrating that epitopes derived from EBNA-1 are presented by a diverse array of HLA alleles. Importantly, EBNA-1-specific CD8+ T cell responses were significantly less frequent in patients with EBV-associated NPC. The frequency of LMP2-specific CD8+ T cells was not significantly different in NPC patients, but the repertoire was narrower compared to healthy donors.

Conclusion: EBNA-1-specific CD8+ T cell responses are frequent in EBV-immune healthy individuals. On the contrary, in EBV-specific T cell lines derived from EBV-immune individuals with NPC, EBNA-1-specific CD8+ T cell responses are significantly less frequent along with a narrower repertoire of LMP2-specific CD8+ T cells. These defects in EBV-specific immunity may be associated with the development or lack of immune control of EBV-associated NPC.

NOTES:

Poster sessions

Session 6: Nasopharyngeal Carcinoma

170 (RegID: 1016)
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NASOPHARYNGEAL CARCINOMA (NPC): MOLECULAR PATHOGENESIS AND CLINICAL INTERVENTION

Posterabstract:
For therapeutic intervention of NPC, we use the NPC cell lines to select a 12-mer specific peptide which can bind specifically to the surface of NPC cells by phage-displayed random peptide library. This peptide has met several criteria for targeted drug delivery into a NPC solid tumor. In vitro experiment the peptide can bind specifically to the cell surfaces of most NPC cell lines and biopsy specimens; the peptide-linked liposome containing fluorescent substance is capable of binding to and translocation across cell membranes; in vivo, this specific peptide can bind and accumulate in the xenograft in SCID mice, but not in normal organs; similarly the peptide-linked liposome carried doxorubicin not only can cause marked cytotoxicity of NPC cells in vitro, in vivo, it can also suppress markedly the xenograft growth in SCID mice without systemic side effect; when the L-peptide is linked with iron oxide nanoparticles, the L-peptide-iron oxide can be used for MR imaging analysis of NPC tumor site in vivo. In conclusion, the novel peptide we identified can be used for targeted chemotherapy with high efficacy and without systemic side effect and for MRI analysis of NPC tumor site in vivo. Application of this peptide-targeted therapy against NPC may let this cancer becomes easier to be controlled.

171 (RegID: 1027)
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MOLECULAR DETERMINANTS OF THE BARF1-HCSF1 INTERACTION

Nicolas Tarbouriech, Tadamasa Ooka, Wim P Burmeister

Posterabstract：

The BARF1 protein is expressed in EBV-associated nasopharyngeal cancer and has an immunosuppressive and tumorigenic role (Wei and Ooka, 1989; Wei et al., 1994). We solved recently its 3D structure (Tarbouriech et al., 2006) which shows a hexameric soluble glycoprotein closely related to CD80, each monomer being composed of 2 immunoglobulin domains. It had been shown that BARF1 binds to hCSF-1, the human colony-stimulating factor 1 (Strockbine et al., 1998). We now want to elucidate the structural basis of this interaction which has to be principally different from the one between hCSF-1 (a 4-helix bundle cytokine) and its natural receptor. For this we developed an efficient production of BARF1 in insect cells using a secreted His-tagged construct we could purify from the culture media. hCSF1 is produced in bacteria as inclusion bodies and renatured. The BARF1-CSF1 complex is readily formed upon mixing of the purified proteins and can be visualized by his-pull down assays and gel filtration. Size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS) confirmed the observed 1:1 stoechiometry observed during the pull down assays. The hexameric form of BARF1 and dimeric form of hCSF1 implies 3 dimers of hCSF1 per hexamer of BARF1. This complex of 300 kDa is now subjected to crystallization trials in order to determine its 3D structure. Understanding the molecular determinants of this interaction will shed some lights on the processes involved in the immunosuppressive action of BARF1.

172 (RegID: 1054)
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MONOCHROMOSOME TRANSFER OF AN INTACT CHROMOSOME 14 SUPPRESSES TUMOR FORMATION IN NASOPHARYNGEAL CARCINOMA

Arthur Kwok Leung Cheung, Hong Lok Lung, Yue Cheng, Eric John Stanbridge, and Maria Li Lung

Posterabstract：

Extensive allelic losses occur on chromosome 14 in a number of human cancers, including nasopharyngeal carcinoma (NPC). In our previous studies using a microcell-mediated chromosome transfer (MMCT) approach, microcell hybrids (MCHs) were established after transfer of an intact human chromosome 14 into the HONE1 NPC cell line. Uniform loss of a commonly eliminated region was mapped by microsatellite typing and fluorescence in situ hybridization (FISH) BAC assays to chromosome regions at 14q11.2-13.1 and 14q32.1. We concluded that hybrid selection and tumor growth in vivo were associated with this selective genomic elimination. Selected genes mapping to these eliminated regions are now the subject of current investigations. To obtain direct conclusive evidence that these regions are indeed responsible for actual tumor suppression, in this current study, new MCH cell lines were established after transfer of chromosome 14, which retained the intact chromosome, as confirmed by microsatellite and FISH assays. These MCH cell lines are tumor-suppressive, when injected into nude mice. After a latency period of 6-8 weeks, tumors reaching the size of around 900 mm3 formed by weeks 8-9, as compared to the recipient HONE1 NPC cell line, which formed tumors of that size by week 5. The increased latency period and decreased size and number of tumors formed in the animals, after addition of the intact chromosome 14 to the HONE1 cells, indicate that a gene or genes present in the transferred chromosome are able to functionally complement the defects present in the NPC cell line. Tumor segregants (TSs) were established from these MCH cell lines after 12 weeks, which allow further molecular genotyping to narrow down the region of interest that presumably is associated with tumor suppression. These studies are ongoing and results indicate that chromosome 14 contains critical regions associated with tumor suppression in NPC.

173 (RegID: 1063)

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IDENTIFICATION OF CANDIDATE TUMOR SUPPRESSOR GENE, MATRIX METALLOPROTEINASE-19 (MMP19), IN NASOPHARYNGEAL CARCINOMA

Chan KC, Ko MY, Yau WL, Lung HL, Sedlacek R, Lerman MI, Zabarovsky ER, Miller LD, Liu ET, Chua DT, Tsao SW, Lung ML

Posterabstract：

Genetic alterations are important for the development of nasopharyngeal carcinoma (NPC). Inactivation of tumor suppressor genes (TSGs) is frequently detected in NPC. BLU/ZMYND10 is a TSG located on 3p21.3, which is frequently inactivated or down-regulated in NPC. Our previous studies demonstrated its tumor suppressive role and provided functional evidence of BLU as a promising TSG in NPC. In order to identify the genes regulated by BLU, gene expression profiles of the tetracycline-regulated BLU stable transfectants and controls were analyzed by competitive hybridization on the 19K oligonucleotide microarray. 12 genes were found to be up-regulated when there was BLU over-expression. Matrix metalloproteinase-19 (MMP19), located on chromosome 12q14, was one of those 12 genes found to be up-regulated by BLU over-expressing transfectants. It was also found to be down-regulated in comparative microarray studies of four NPC cell lines versus the immortalized normal nasopharyngeal epithelial cell line, NP460. Down-regulation of MMP19 was observed in 7/7 (100%) NPC cell lines and down-regulated in 12/30 (40%) NPC biopsies. Reduction of colony forming ability was observed in NPC cell lines transfected with MMP19. The nude mouse tumorigenicity assay of MMP19 stable transfectants showed over-expression of MMP19 suppressed tumor formation, while loss or down-regulation of the transgene was observed in tumors derived from tumorigenic transfectants. MMP19 is a protease known to be involved in the breakdown of extracellular matrix components. Its involvement in several human cancers indicates a potentially important role for this gene in cell proliferation, migration, and adhesion. Thus, it is expected to be an important potential candidate TSG in NPC, which may contribute to tumor invasion. Further functional studies are underway focused on investigating the tumor suppressive role of MMP19in NPC.

174 (RegID: 1069)

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SPLUNC1 POLYMORPHISMS ASSOCIATED WITH NASOPHARYNGEAL CARCINOMA SUSCEPTIBILITY IN MALAYSIAN CHINESE

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Posterabstract：

Nasopharyngeal carcinoma (NPC) is a malignancy of the head and neck region that occurs in the nasopharynx tissue. NPC rarely occurs in most parts of the world, but it is common in China, Taiwan and Southeast Asia including Thailand, Vietnam, Indonesia, Malaysia and Singapore. In West Malaysia, NPC is the second and twelfth most common cancer among men and women respectively. The fact that Malaysian Chinese shows higher incidence of NPC compared to other races revealed that genetic susceptibility might be a crucial etiologic factor. In this study, we aimed to investigate the association of SPLUNC1 genes with NPC in Malaysian Chinese. We conducted a case-control study by genotyping eight tagged-single nucleotide polymorphisms (tSNPs) within this gene in 159 NPC cases and 408 controls subjects. Here, we reported that four tSNPs were significantly associated with NPC. Among these 4 tSNPs, one was most significantly associated with NPC [p =0.0003, OR=2.00, 95% CI =1.38-2.89]. Hence our result suggests that SPLUNC1 polymorphisms may contribute to the susceptibility to NPC.

175 (RegID: 1132)
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INTEGRATIVE CANCER EPIGENTICS IDENTIFIES A NOVEL RAB GTPASE AS A FUNCTIONAL TUMOR SUPPRESSOR FREQUENTLY SILENCED IN NASOPHARYNGEAL AND MULTIPLE OTHER CARCINOMAS

Jisheng Li, Jianming Ying, Hongyu Li, Ka Man Ng, Qian Tao

Posterabstract:

Epigenetic silencing of tumor suppressor genes (TSGs) through methylation of promoter CpG islands (CGI) contributes to the pathogenesis of multiple cancers including nasopharyngeal carcinoma (NPC). TSGs often locate at frequently deleted chromosome regions. We utilized an integrative genomic epigenetic approach by combining high-resolution array comparative genomic hybridization (aCGH) with epigenetic profiling to screen for epigenetically inactivated TSGs. A 1.5-Mb deletion at 11q22 was refined by aCGH in multiple tumor cell lines, indicating the presence of critical TSGs. Semi-quantitative RT-PCR analysis revealed that TUSC12 gene was downregulated in multiple carcinoma cell lines, in contrast to its broad expression in normal adult and fetal tissues. TUSC12 belongs to the RAB GTPase family, which is involved in the regulation of vesicular membrane traffic and associated with multiple human diseases including cancer. Frequent promoter methylation of TUSC12 was detected in multiple carcinoma cell lines including esophageal, nasopharyngeal, colon, breast, hepatocellular and gastric carcinomas, as well as primary tumors, but not in immortalized normal epithelial cell lines. Furthermore, transcriptional silencing of TUSC12 could be reversed by pharmacologic demethylation with 5-aza-2'-deoxycytidine, indicating a direct epigenetic inactivation. Ectopic expression of TUSC12 in tumor cells lacking its expression dramatically inhibited their clonogenicity, confirming the tumor suppressor function of this gene. Thus, a RAB GTPase TUSC12 was identified as a novel TSG with frequently epigenetic silencing in multiple carcinomas.

176 (RegID: 1132)
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EPIGENETIC MECHANISM OF RHOA SIGNALING ACTIVATION IN HUMAN CANCERS THROUGH SILENCING A NOVEL NEGATIVE REGULATOR RHOA-GAP - DLC1L1

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Posterabstract：
Aberrant activation of RhoA and Ras signaling is common in human cancers including nasopharyngeal carcinoma (NPC), while the molecular mechanisms of this aberrant signaling are diverse. Epigenetic inactivation of tumor suppressor genes (TSGs) through promoter CpG methylation is commonly involved in tumorigenesis, which can be used as a biomarker for TSG identification and cancer diagnosis. We used a novel uracil-DNA glycosylase-based CpG methylation subtraction to search for epigenetically-silenced genes in HCT116 cells deficient in DNMT1 and DNMT3b (DKO cells). Among the upregulated genes identified, we focused on DLC1L1, encoding a RhoA GTPase activator domain protein (GAP). Semi-quantitative RT-PCR showed that DLC1L1 is ubiquitously expressed in all normal tissues examined, while silenced in most carcinoma cell lines including NPC due to promoter methylation, and unmethylated in immortalized normal epithelial cell lines. Expression of DLC1L1 was dramatically increased upon pharmacological demethylation with 5-aza-2’-deoxycytidine or genetic demethylation (DKO), indicating methylation directly mediated repression. Aberrant methylation was frequently detected in primary carcinomas, but rarely in paired normal samples. Ectopic expression of DLC1L1 in silenced tumor cells resulted in substantial inhibition of tumor cell colony formation. The RhoA pull-down assay demonstrated a remarkable reduction of RhoA activity in the DLC1L1 transfected cells. The tumor suppression function depends on GAP activity of DLC1L1, as inactivating mutants at the GAP domain (R399A, del) abolished the tumor suppression. Furthermore, DLC1L1 but not its inactivating mutants, inhibited Ras-mediated oncogenic transformation. These results suggest that DLC1L1 exerts its tumor suppressor function as a novel GAP for RhoA, acting as a TSG to suppress tumor cell growth and transformation.

177 (RegID: 1132)
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CHD5 IS A NOVEL 1P36 TUMOR SUPPRESSOR INVOLVED IN HETEROCHROMATIN SILENCING, BEING FREQUENTLY SILENCED BY CPG METHYLATION IN MULTIPLE TUMORS

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Posterabstract：
Promoter CpG methylation inactivates tumor suppressor genes (TSG), which is involved in tumorigenesis and is an epigenetic biomarker for novel TSG identification. We identified *CHD5* as a silenced candidate TSG through *in silico* differential gene expression displayer (DGED) subtraction analysis (Cancer Genome Anatomy Project) in nasopharyngeal (NPC). *CHD5* is also located within a 1p36 deletion refined by our 1-Mb array-CGH in NPC and esophageal carcinoma cell lines. CHD5 is a member of the chromodomain superfamily of chromatin remodeling proteins, containing two Zn-binding plant homeodomain (PHD) fingers, a SNF2-like ATPase/Helicase domain and a DNA binding SAND domain. We found that *CHD5* is expressed in most normal tissues, but frequently downregulated or silenced in multiple carcinoma and lymphoma cell lines (25-100%). The *CHD5* promoter is a typical CpG island and methylated in all silenced cell lines. Aza treatment (or combined with TSA) demethylated and activated *CHD5* expression in silenced cell lines. *CHD5* is also methylated in 42% (20/48) of primary NPC and 54% (37/68) of gastric carcinomas, but less frequently in other carcinomas. CHD5 was found to be a nuclear protein forming patchy granules, and colocalized with HP1 to heterochromatin foci, suggesting that *CHD5* is involved in heterochromatin silencing. Ectopic expression of *CHD5* resulted in strong inhibition of tumor cell clonogenicity, in both anchorage-dependent and -independent manners, suggesting that CHD5 functions as a tumor suppressor in tumor cells. Considering the reported rarity of *CHD5* mutations in human cancers, epigenetic disruption of *CHD5* represents the predominant alteration of this TSG in tumors. *CHD5* is thus the second critical 1p36.3 tumor suppres

178 (RegID: 1142)
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EFFECT OF DNAZYMES TARGETING AKT1 ON CELL PROLIFERATION AND APOPTOSIS IN NASOPHARYNGEAL CARCINOMA

YangLifang

Posterabstract:

The phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is important in nasopharyngeal carcinoma (NPC) pathogenesis. Activated PI3K and its downstream target Akt are concernful signaling molecules and key survival factors involved in the control of cell proliferation, apoptosis and oncogenesis. The protein kinase Akt1, one of the Akt family, is involved in several signaling pathways for tumor development and progression, suggesting that Akt1 might be an interesting target for a molecular tumor therapy. DNAzyme is a single-stranded DNA catalyst that can be engineered to bind to their complementary sequence in the target mRNA and cleave the mRNA at predetermined phosphodiester linkages. In this study, based on the analysis of sequences, thermodynamics and site distribution within the Akt1 gene, five DNAzymes were designed and synthesized. We found that the DNAzyme, namely DZ2, strongly inhibited Akt1 mRNA and protein expression in the NPC cells line CNE1-LMP1, and showed that the DZ2 strongly blocked the cellular signal transduction pathways that were abnormally activated by Akt1. This effect was shown to be associated with inhibition of cell proliferation, stimulation of apoptosis and inhibition of xenograft growth in nude mice by suppression of the Bcl-2 and increase of Bax expression. Thus, Akt1 targeting DNAzymes, DZ2, can inhibit multiple key tumorigenic processes in vitro and in vivo and may serve as useful anti-cancer agent in NPC.

179 (RegID: 1503; 1504; 1506; 1507; 1510)

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RECURRENT CHEMICAL REACTIVATIONS OF EBV PROMOTES GENOME INSTABILITY AND CHROMOSOMAL ABERRATIONS OF NASOPHARYNGEAL CARCINOMA CELLS

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Abstract:

Nasopharyngeal carcinoma (NPC) is an endemic malignancy prevalent in southern Chinese males. Epidemiological studies have associated this disease closely with Epstein-Barr virus (EBV) infection. It also has been proposed that chemicals in herbal medicines or diet may induce EBV reactivation and lead to NPC. Because of the lack of an appropriate model system, this hypothesis has not been seriously tested and the mechanism has not been elucidated. Genomic instability is considered to contribute to the development of human cancers and has been found to occur in many NPCs. Therefore, in this study, we tried to elucidate whether EBV reactivation by chemicals promotes genomic instability of NPC cells. NPC cell lines latently infected with EBV, NA and HA, and the corresponding EBV-negative NPC cell lines, NPC-TW01 (TW01) and HONE-1, were treated with 12-O-tetradecanoylphorbol-13-acetate (TPA) and sodium n-butyrate (SB). Increases in genomic instability were determined by the formation of micronuclei (MN). Double strand breaks (DSB) in DNA were detected by g-H2AX staining. A single treatment with TPA/SB revealed that formation of MN increased in NA and HA cells but not in TW01 and HONE-1 cells. However, 15 recurrent reactivations of EBV in NA and HA cells resulted in a marked increase in the accumulation of genomic instability, as revealed by the formation of MN and chromosomal aberrations. Transwell assay of TW01 and NA cells revealed a profound increase in the invasiveness of the repeatedly reactivated NA cells but not TW01 cells. These data show that EBV reactivations may result in DNA damage and consequent genomic instability. With recurrent reactivations these effects accumulate and may lead to a more malignant phenotype of NPC.

180 (RegID: 1601; 1602)

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PROFILING AND PATHWAY ANALYSIS OF MICRORNAS ALTERED IN NASOPHARYNGEAL CARCINOMA

HC Chen, GH Chen, YH Chen, WL Liao, CY Liu, KP Chang, YS Chang and SJ Chen

Posterabstract:

MicroRNAs (miRNAs) are a family of small non-coding RNA molecules of about 20-23 nucleotides in length, which negatively regulate protein-coding genes at post-transcriptional level. Using a stem-loop real-time-PCR method, we quantified the expression levels of 270 human miRNAs in 13 nasopharyngeal carcinoma sample (NPC) and 9 adjacent non-tumor tissue (NT) and identified several miRNAs whose expression levels were significantly altered in NPC samples. Several known oncogenic miRNAs, including miR-17-92 cluster and miR-155, are among the miRNAs up-regulated in NPC. Tumor suppressor miRNAs, including miR-34 family, miR-143, and miR-145, were significantly down-regulated in NPC. To explore the roles of these dysregulated miRNAs in the pathogenesis of NPC, a computational analysis was performed to predict the pathways collectively targeted by the 22 most significantly down-regulated miRNAs.

Several biological pathways and networks that are well-characterized in cancer were significantly targeted by the down-regulated microRNAs. These pathways include Wnt pathways, growth factor regulated G1-S cell cycle progression, VEGF signaling pathways. The expression levels of several genes involved in G1-S progression, cyclin D2, cyclin E2, CDC25A, were inversely correlated with the levels of down-regulated microRNAs in NPC tissues. We examined the microRNA binding sites on the 3'UTR of cyclin E2 and found conserved binding sites for miR-34c, miR-200a, and miR-9. Expression levels of cyclin E2 in NPC tissue show significant inverse correlation with these three microRNAs. These results indicate that these down-regulated miRNAs coordinately regulate several oncogenic pathways and provide a experimentally testable hypothesis regarding collective regulatory function of microRNAs in cancer.

181 (RegID: 1706; 1785)

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EPSTEIN-BARR VIRUS ENCODED LATENT MEMBRANE PROTEIN 1 AND 2A INDUCED MICRORNA-155 EXPRESSION IN NASOPHARYNGEAL CARCINOMA

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Posterabstract:

MicroRNAs (miRNAs) are a new class of non-coding RNAs , which can exert their gene regulatory activity by specific mRNA degradation or translational inhibition. The objectives of this project are to shed light on the contribution of miRNAs in EBV oncogenesis in NPC and to explore if they can be used as molecular markers for diagnosis and prognosis prediction to improve the clinical handling of NPC patients.

Differential expressed miRNAs and mRNAs regulated by LMP1 and LMP2A were investigated by screens with miRNAs microarray and Affymetrix Gene Chip in NPC TW03 cells respectively. Quantitive Realtime PCR was used to validate these array data. Compared with vector control, miR-155 expression were increased in two LMP1-CNE1 stable clones (158.24 ± 25.23 fold; 168.89±30.87 fold), one LMP1-TW03 stable clone (42.56 ± 9.87 fold), and in one LMP2A-TW03 stable clones (2.16 ± 0.41 fold). With TFSEARCH NF-kB and AP-1 binding site were found in the promoter region of pri-miR155 (BIC). For targets prediction of miR-155, four algorithms - miRanda (John et al., 2004), TargetScan (Lewis et al., 2005), PicTar (Krek et al., 2005) and miRBase (Enright et al., 2003) were used and we chose 47 common putative targets which were in at least 3 algorithms. Combined with the mRNAs array data, we found that BACH1, CARHSP1, HIVEP2, JMJD1A, TLE4, FGF7, USP48 and FBX011, which are putative targets of miR-155, were downregulated by LMP1 and LMP2A in TW03 cells.

The functional significance of selected miRNAs will be verified by switching on or off their expression using mimic or anti-sense of miR-155 followed by assessement of their targets and biological effects. Finally, we will use in situ hybridization (ISH) or immunohistochemistry (IHC) to detect the expression of selected miRNAs and their targets in NPC biopsy and evaluate the diagnostic and prognostic predictive value.

182 (RegID: 1882)
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LATENT MEMBRANE PROTEIN 1 SUPPRESSES RASSF1A EXPRESSION, DISRUPTS MICROTUBULE STRUCTURES AND INDUCES CHROMOSOMAL ABERRATIONS IN HUMAN EPITHELIAL CELLS

Cornelia Man, Jack Rosa, Leo Tsz On Lee, Vien Hoi Yi Lee, Billy Kwok Chong Chow4, Kwok Wai Lo, Stephen Doxsey, ZG Wu, YL Kwong,Annie LM Cheung1, Sai Wah Tsao

Posterabstract:
EBV infection is closely associated with nasopharyngeal carcinoma (NPC) and can be detected in early premalignant lesions of nasopharyngeal epithelium. The latent membrane protein 1 (LMP1) is an oncoprotein encoded by the Epstein-Barr virus and is believed to play a role in transforming premalignant nasopharyngeal epithelial cells into cancer cells. RASSF1A is a tumour suppressor gene commonly inactivated in many types of human cancer including nasopharyngeal carcinoma. In this study, we report a novel function of LMP1, in down-regulating RASSF1A expression in human epithelial cells. Down-regulation of RASSF1A expression by LMP1 is dependent on the activation of intracellular signaling of NF-κB involving the C-terminal activating regions (CTARs) of LMP1. LMP1 expression also suppresses the transcriptional activity of the RASSF1A core promoter. RASSF1A stabilizes microtubules and regulates mitotic events. Aberrant mitotic spindles and chromosome aberrations are reported phenotypes in RASSF1A inactivated cells. In this study, we observed that LMP1 expression in human epithelial cells could induce aberrant mitotic spindles, unstable interphase microtubules and aneuploidy. LMP1 expression could also suppress microtubule dynamics as exemplified by tracking movements of the growing tips of microtubules in live cells by transfecting EGFP-tagged EB1 into cells. The aberrant mitotic spindles and interphase microtubule organization induced by LMP1 could be rescued by transfecting RASSF1A expression plasmid into cells. Down-regulation of RASSF1A expression by LMP1 may facilitate its role in transformation of premalignant nasopharyngeal epithelial cells into cancer cells.

183 (RegID: 1931; 1932)

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LONG TERM IN VITRO CULTURE OF NASOPHARYNGEAL CARCINOMA CELLS FROM A XENOGRAFT

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Posterabstract:

In vitro culture of cancer cells is highly useful for cancer biology and other functional studies. The limited availability of established cell lines for nasopharyngeal carcinoma (NPC) is an obstacle for further research on the cancer. In addition, the authenticity of several cell lines, used by researchers in studies on nasopharyngeal carcinoma, has been questioned. This has made the establishment of new NPC cell lines even more critical.

An EBV-positive nasopharyngeal xenograft, previously derived from metastatic nasopharyngeal carcinoma tissue in a patient who had received chemotherapy and radiotherapy, has been propagated in nude mice. In vitro culture of these cells has been known to be difficult and frequently limited to short term cultures.

Here, we report long term in vitro propagation of nasopharyngeal carcinoma cells from the xenograft. The cells have been continuously propagated in vitro for at least 3 months. The cells retained their ability to grow on soft agar. These cells are potentially useful for functional studies which require in vitro culture.

NOTES:

Poster sesions

Session 7: Burkitt's Lymphoma

184 (RegID: 1156)
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EBV-ASSOCIATED BURKITT'S LYMPHOMA (BL) IN MALAWI, EAST AFRICA: GENE EXPRESSION, DIAGNOSIS AND THERAPY

Beverly E. Griffin1, 4, Susan van Noorden2, I.A. Lampert2, S-A Xue3 and J.A. Phillips4. Imperial College School of Medicine at St. Mary's1 and at Hammersmith Hospitals2, University College Medical School3, London U.K., and the BL unit, Kamuzu Central Hospital, Malawi4

Posterabstract:
A major study on BL in Malawi, East Africa, over the past twenty years, will be presented. Briefly: this EBV-associated malignancy remains the major childhood cancer seen in Malawi, and patient numbers are high; about 60% of confirmed BL cases, whether facial or abdominal, respond to classical multidose treatment with cyclophosphamide; therapy for the others is generally unsuccessful. Qualitatively, EBV gene expression varies among the tumours. Our chief aims over the past twenty years have focused on: trying to understand the differences, at a molecular level, between BL patients that respond to the chemotherapy and those that do not; seeking markers to aid in determining optimal management of patients; and, identifying an alternative therapeutic regiment that can be used to combat the tumour in non-responding patients. We will present data on all three topics.
Our current focus for alternative treatment of the non-responding tumours is based on a small preliminary pilot study of children with gross, cyclophosphamide-resistant multiple lesions. Sodium phenylbutyrate), a histone deacetylase (HDAC) inhibitor, was added to the treatment protocol for these patients. This HDAC was chosen for its simple chemistry which suggests that it could ultimately be made cheaply for Africa, its multiple functions, particularly those associated with promotion of cell differentiation, and its established safety in the treatment of another disease (urea disorder) of children. Our data, to be presented, are encouraging, proving positive for half the treated children. They further highlight differences that exist, moreover, among the tumour populations.

185 (RegID:1188)

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CLINICOPATHOLOGICAL FEATURES AND EPSTEIN-BARR VIRUS INFECTION STATUS OF BURKITT LYMPHOMA IN GUANGZHOU

HUANG Yu-hua, WU Qiu-liang, ZONG Yong-sheng，FENG Yan-fen，LIANG Jian-zhong , HOU Jing-hui , SHAO Qiong，FU Jia

Posterabstract:

Background To investigate the clinical presentation, morphological features, immunophenotype and Epstein-Barr virus (EBV) infection status of Burkitt lymphoma (BL) in Guangzhou, a prevalent area of EBV infection. Methods The clinical data of 21 Burkitt lymphoma patients were reviewed. A panel of immunohistochemical staining and EBV-encoded small RNAs (EBERs) in situ hybridization was performed. Results (1) Twenty-one BLs could be confirmed by reviewing 2,416 non-Hodgkin lymphomas (21/2,416, 0.87%) during the period of January 1, 2000 to October 30, 2007. (2) Male to female ratio was 4.25 (17/4). The median age of patients was 23 year-old. The lymph node(s) involvement was found in 19 of 21 cases (90.48%). Multiple sites had been involved in 16 cases (76.19%); and 12 patients (57.14%) were attributed to clinical stage III/IV. The consequence of 15 follow-up patients with chemotherapy or resection plus chemotherapy was not satisfactory. (3) Twenty cases showed the morphology of classical BL, and 1 case was the variant of BL with plasmacytoid differentiation. The Ki-67 proliferation index reached nearly 100% in all cases. (4) The crucial immunophenotype of these 21 BLs was sIgM+/CD20+/CD10+/BCL-6+/BCL-2- (or BCL-2+%<Bcl-6+%)/MUM1- (or MUM1+%<BCL-6+%)/TdT-. A few neoplastic cells (3~20%) could express CD5 in 11 out of 20 available cases. (5) The p53 protein was overexpressed in 10 tumours (47.62%). (6) The neoplastic cells in 6 out of 21 BLs (28.57%) harbored EBV, expressing EBNA1 and EBERs, but not LMP1. There were no significant differences either in morphology or immunophenotype between EBV-positive and EBV-negative cases. Conclusions The sporadic BL is uncommon in Guangzhou. The most patients are male children and young adults. This tumour is a highly aggressive lymphoma, and lymph node(s) involvement is not infrequent. The vast majority of cases showed the morphology and immunophenotype of classical BL. About half of the cases exhibit p53 overexpression. The 28.57% of cases is associated with EBV harboring, being type I latency.

186 (RegID: 1777)
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EBV LATENCY PATTERN DETERMINES THE PRECISE B CELL DIFFERENTIATION STATUS OF BL

Gemma L. Kelly1, Wendy A. Thomas1, Julianna Stylianou1, Wenbin Wei1, Paul Kellam2, Jane Rasaiyaah2, Wolfgang Hammerschmidt3, Marcus Altmann3, Alan B. Rickinson1, Martin Rowe1 and Andrew I. Bell1
1 Cancer Research UK Institute for Cancer Studies, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK. 2 Virus Genomics and Bioinformatics Group, Division of Infection and Immunity, University College London, London W1T 4JF, UK. 3 GSF-National Research Center for Environment and Health GmbH, Institute of Clinical Molecular Biology and Tumour Genetics, Marchioninistr. 25, 81377 München, Germany

Posterabstract:
The pathogenesis of the germinal centre (GC)-derived tumour, Burkitt lymphoma (BL) is attributed to two factors, a chromosomal translocation leading to deregulated c-myc and the presence of EBV. The contribution of each of these factors and how they complement one another remains unclear. Interestingly, EBV's full growth transforming program is never detected in BL and this is thought to be due to antagonism between it and the c-myc-driven growth program. To this end, BL cells carry EBV in one of two restricted forms of latency, both of which are compatible with high level c-myc expression. Most BLs display a Latency I infection, characterised by expression of EBNA1 only, while a subset of BLs show a Wp-restricted form of latency which involves expression of EBNAs 1, 3A, 3B, 3C, tr-LP and BHRF1.

In order to address the effects of EBV latency on the BL cell phenotype we carried out microarrays on a panel of BL cell lines displaying different associations with EBV, namely they were EBV-negative, Latency I or Wp-restricted. This revealed that Wp-restricted BLs, when compared to EBV-negative and Latency I BLs, show a downregulation of the transcription factor BCL6, which is normally expressed highly in GC B cells and BL cells Using quantitative RT-PCR assays we found that Wp-restricted BLs not only show a downregulation in BCL6, but also display an upregulation of the plasma cell markers BLIMP1 and IRF4. Using recombinant viruses we have recapitulated Wp-restricted latency and shown that these effects are a direct consequence of EBV antigen expression and not the cellular origin of the tumour progenitor cell. In conclusion, different patterns of EBV latency can influence the differentiation status of the BL cell, with Wp-restricted latency imposing a more differentiated state on the cell which most closely resembles B cells committed to exiting GCs.

187

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THE PREVALENCE OF EBV INFECTION IN DIFFERENT TYPES AND SITES OF LYMPHOMAS

GAN Runliang (甘润良), PENG Jinyun, TANG Yunlian, HE Jie, HE Rongfang
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Grant support: National Natural Science Foundation of China (30801335), Hunan Provincial Natural Science Foundation (08JJ3034), and the Opening Foundation of Key Lab of Virology of China Science Academy (2005008).

Posterabstract:

Epstein-Barr virus (EBV) is a kind of DNA virus related to the occurrence of some human malignancies. The aim of this study was to investigate the association of several types of malignant lymphomas in different anatomical sites with EBV infection status. 127 cases of paraffin-embedded tissues of malignant lymphomas were composed of 60 lymphomas in nasal cavity and nasopharynx, 30 lymphomas in stomach and 37 lymphomas in superficial lymphnodes. There were 19 cases of Hodgkin's disease (HD), and 108 cases of non-Hodgkin's lymphoma (NHL). By using S-P immunohistochemistry, all of 108 non-Hodgkin's lymphomas were divided into three immunophenotypes: B-cell lymphomas, T-cell lymphomas and NK/T-cell lymphomas according to their different immune markers CD20, CD79a, CD45RO, CD3 and CD56. In situ hybridization was employed to detect the EBV-encoded small RNA (EBER) in tumor cells. There were 46 EBER positive cases in all of 108 cases of NHL including at nasal cavity and nasopharynx, stomach and superficial lymph nodes. EBER positive rate of NHL was 42.6%. EBER-positive rate of NHL in nasal cavity and nasopharynx was 58.3% (35/60 cases). NK/T-cell lymphoma was 29 cases, including 19 EBER positive cases. The EBER-positive rate of NK/T-cell lymphomas was 65.5% (19/29 cases). B-cell lymphoma was 31 cases, including 16 EBER positive cases. EBER-positive rate of B-cell lymphoma in nasal cavity and nasopharyngx was 51.6% (16/31 cases). EBER-positive rate of primary gastric NHL was 30% (9/30 cases). There were 28 cases of gastric B-cell lymphomas, including 9 EBER positive cases. EBER positive rate of gastric B-cell lymphomas was 32.1% (9/28 cases). There were 2 cases of gastric T-cell lymphoma, which was EBER negative. EBER positive rate of NHL in superficial lymph nodes was 11.1% (2/18 cases), including one EBER positive case in 8 cases of T-cell lymphomas and one EBER positive case in 10 cases of B-cell lymphomas. In addition, EBER positive rate of HD in superficial lymphnodes was 26.3% (5/19 cases). The present detection showed that EBV infection rate (42.6%) of NHL was a little higher than that (26.3%) of HD, however, their difference was not significant ($p>0.05$). EBER positive rate of NHL in nasal cavity and nasopharyngx was higher than that of NHL in stomach and in the superficial lymphnodes ($p<0.05$). These findings suggest that different types of human lymphomas are strongly associated with EBV infection, and EBV positive rate of lymphomas is also related with their sites.

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HUMAN-DERIVED IGG LEVEL AS A INDICATOR FOR EBV-ASSOCIATED LYMPHOMA MODEL IN HU-PBL/SCID CHIMERA

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Posterabstract:
EB virus has a close association with various types of human lymphomas. Animal model are essential to elucidate the pathogenesis of human EBV-associated lymphomas. The aim of the present study was to evaluate the association between human IgG concentration and EBV-associated lymphoma development in hu-PBL/SCID mice. Human peripheral blood lymphocytes (hu-PBL) from EBV-seropositive donors were inoculated intraperitoneally into SCID mouse. Immunohistochemical staining was used to examine differentiated antigens of tumor cells. IgG concentrations in the serums of 12 SCID mice were measured by unidirectional immunodiffusion assay. 21 out of 29 mice (72%) developed tumors. Immunohistochemical staining showed that all induced tumors were LCA (leukocyte common antigen) positive, B-cell marker (CD20) positive, and T-cell markers (both CD3 and CD45RO) negative. From these morphological and immunohistochemical features, those tumors can be diagnosed as human B-cell lymphomas. In situ hybridization exhibited resultant tumor cells had EBV encoded small RNA-1 (EBER-1). Human-derived IgG could be found in the serum of SCID mice on the 15th day following hu-PBL transplantation, and IgG levels increased with the tumor development in 6 hu-PBL/SCID chimeras. Human IgG concentration in sera of hu-PBL/SCID chimeras was also related to the IgG level in original donor's serum. Intraperitoneal transfer of hu-PBLs from EBV+ donors into SCID mice leads to high human IgG levels in mouse serum and B cell lymphamas. Our findings suggest that increasing levels of human-derived IgG in peripheral blood of hu-PBL/SCID mice could be used to monitor EBV-related human B-cell lymphoma development in experimental animals.

NOTES:

Poster sessions

Session 8: PTLD & Other Malignancies

189 (RegID: 1071; 1072)

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AUTOCRINE FUNCTION OF INTERFERON-GAMMA-INDUCIBLE PROTEIN-10 (IP-10) IN NASAL NK/T CELL LYMPHOMA

Shigetaka Moriai, Miki Takahara, Kan Kishibe, Hideyuki Ishii, Yasuaki Harabuchi

Posterabstract:

Nasal NK/T-cell lymphoma is characterized by progressive necrotic lesions with infiltrative tumor cells in the nasal cavity. Prognosis of the lymphoma have very poor with progressive infiltration of lymphoma cells in local lesion and/or distant metastasis. Therefore, it is necessary for the treatment of the lymphoma to understand of the tumor characteristics. Previously we reported that the lymphoma cells are infected with Epstein-Barr virus (EBV), and that EBV is related to pathogenesis of the lymphoma through cytokines such as IL-9 and IL-10. Recently, many studies describing the contribution of chemokines for tumor development including proliferation, migration, and angiogenesis are reported. However, there are few reports about the relationship between chemokines and Nasal NK/T-cell lymphoma. In this study, we examined contribution of the chemokine for development of nasal NK/T cell lymphoma.

Analysis of Human Chemokine protein Array (RayBio) showed that EBV positive NK lines such as SNK6 and KAI3 produced IP-10 (CXCL10), but EBV negative NK lines did not. These results were confirmed by Reverse-transcription (RT)-PCR analysis. RT-PCR and flow cytometric analysis revealed that CXCR3, receptor for IP-10, was also expressed in NK lines. Immunohistochemical analysis demonstrated that IP-10 and CXCR3 expression was detected in tumor cells of biopsy samples from lesions of nasal NK/T-cell lymphoma. These results indicated that some autocrine functions of IP-10 took place in the lymphoma. MTS assay showed that IP-10 did not affect cell proliferation; however, Matrigel assay revealed that IP-10 enhanced invasiveness of the SNK6 cells in autocrine manner. Because the local invasion is known to be one of the prognostic factors in the lymphoma, our findings that IP-10/CXCR3 loop may enhance invasiveness of tumor cells create an opportunity of new treatment by blocking the autocrine loop.

190 (**RegID: 1159; 1030)**

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EXPRESSION OF METALLOELASTASE IN NASAL NATURAL KILLER/T-CELL LYMPHOMA CELL LINES

Kishibe K, Nagato T, Moriai S, Ishii H, Takahara M, Bandoh N,
Harabuchi Y

Abstract:

Nasal natural killer (NK)/T-cell lymphoma has distinct clinical and histologic features and is characterized by a poor prognosis and progressive necrotic lesions with tumor and inflammatory cell infiltrations in the nasal cavity, nasopharynx, and palate. Histologically, the features of this lymphoma include angiocentric and polymorphous lymphoreticular infiltrates. Recent reports suggest that this lymphoma may be derived from NK or gdT-cell lineages, both of which express the NK cell marker, CD56. In this study, we examined the genes expressed by SNK-6, SNK-1 and SNT-8 cells, which were established from nasal NK/T-cell lymphomas, and found that metalloelastase (ME) was specifically expressed in SNK-6 and SNK-1, which have an NK-cell phenotype. ME is a family of extra cellular matrix-degrading enzymes. ME degrades different substrates among which are elastin, laminin, type-IV collagen, fibronectin and casein. About malignant disease, ME is expressed in malignant cells such as skin cancer, astrocytomas, glioblastomas, hepatocellular cancer, and pancreatic cancer. We report the meanings of expression of ME in nasal NK/T-cell lymphoma.

191 (RegID: 1190)

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EBV-POSITIVE NK/T-CELL LYMPHOMA OF THE NASOPHARYNX

HUANG Yu-hua, WU Qiu-liang, ZONG Yong-sheng，FENG Yan-fen，HOU Jing-hui, SHAO Qiong，FU Jia

Posterabstract:

Background and objective The NK/T-cell lymphoma, nasal type is closely associated with Epstein-Barr virus (EBV) infection. We investigated the frequency of this tumour in Guangzhou and its clinicopathologic features. Methods Nineteen subjects suffered from extranodal nasopharyngeal NK/T cell lymphoma were collected from the file of Department of Pathology, Cancer Center of Sun Yat-sen University. A panel of immunohistochemical stains and EBV-encoded small RNAs (EBERs) in situ hybridization were performed. Results The NK/T-cell lymphoma, nasal type occurs 18.63% (19/102) in all nasopharyngeal non-Hodgkin lymphomas; and most of the nasopharyngeal T-cell lymphomas (19/24, 79.17%) were the NK/T-cell lymphoma, nasal type. The ratio of male to female was 1.71：1（12:7）; and the median age was 50 year-old. The great majority of patients （16/19, 84.21%）, when they visited the medical doctors were at the early clinical stages (stage I/II). The primary lymphoma lesion of 13 patients was confined in the nasopharynx. The lymphoma lesion of 5 patients had extended to the parapharyngeal space (1 case), oropharynx (1 case), cranial nerves (1 case), or cranial bone (2 case); and the bone marrow had been involved only in 1 case. The serum antibody level against EBV was slightly elevated. Among the 12 patients with follow-up, 6 ones are alive for 3~52 months up to date and the other 6 patients has been died 3~39 months after completing the therapy. The EBERs-positive neoplastic cells were found in all of the 19 cases, and LMP1 expression occurred in 8 cases (42.11%, 8/19). Two major immunophenotypic subtypes, namely, EBV+/CD56+/TIA+ (10 cases) and EBV+/CD56-/TIA+ (9 cases) could be recognized. Conclusions The patients with extranodal NK/T-cell lymphoma, nasal type primarily originated from the nasopharynx in Guangzhou are all EBV associated. The EBV infection is type Ⅱ latency, and LMP1 expression rate reached 42.11%. Their serum antibody level is elevated slightly. There are two major immunophenotypic subtypes, namely EBV+/CD56+/TIA1+ and EBV+/CD56-/TIA1+, implicating that this tumour may be originated from mature NK cell or NK-like cytotoxic T-cell.

192 (RegID: 1194; 1199)
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INFECTION OF T-LYMPHOCYTES IN EPSTEIN-BARR VIRUS-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN OF NON-ASIAN ORIGIN

Karin Beutel, Ute Gross-Wieltsch, Thomas Wiesel, Udo Zur Stadt, Gritta Janka, Hans-Joachim Wagner

Posterabstract:
Epstein-Barr virus (EBV) is one of the most frequent triggers of hemophagocytic lymphohistiocytosis (HLH). Ectopic infection of T cells in EBV-associated HLH (EBV-HLH) has mainly been described in Asian patients. We provide clinical and virological data of 13 non-Asian children with EBV- HLH. Children with underlying immunodeficiencies as well as otherwise healthy individuals were affected. Elevated EB viral load was detected by real-time polymerase chain reaction in plasma and peripheral blood mononuclear cells. Serial measurements reflected the clinical course of the disease. Cell-type specific viral load was determined in seven patients and revealed EBV-infection of T cells in all of them. In contrast to the reported Asian patients a significant viral load was also found in B cells. Evaluation of cell-type specific infection should be considered when targeted therapy is applied.

193 (RegID: 1195)
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A UNIQUE CASE OF PTLD AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION FOR BCL-ABL POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA

Stephanie Volke, Olga Moser, Wilhelm Wößmann , Andreas Schulz, Birgit Bassali, Peter Bader, Alfred Reiter, Hans-Joachim Wagner

Posterabstract:
We report a unique presentation of an Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) in a 13-year old boy who underwent two subsequent bone marrow transplantations (BMT) from two different donors for BCR-ABL positive acute lymphoblastic leukaemia (ALL) and relapse of the leukaemia, respectively.
Case report: The boy was diagnosed of a BCR-ABL-positive ALL at the age of one year. After remission induction, he received a BMT from HLA-identical brother. At the age of 10 years he suffered a first relapse of the BCR-ABL-positive ALL. He was treated with chemotherapy, imatinib, and donor lymphocyte infusions (DLI) and achieved 2nd remission, but developed chronic graft-versus-host disease (GvHD). The 2nd relapse of the ALL 2 years later responded to imatinib, but progressed within 6 months. Following remission induction, the patient received a 2nd BMT from an 8/10 matched unrelated female donor after TBI-based myeloablative conditioning. The BMT was complicated by cytomegalovirus disease leaving a poor graft function. 4 months after the 2nd BMT the patient presented with fever, painful swelling of tonsils, and marked eosinophilia. A biopsy proved an EBV-induced PTLD of the tonsils. The patient was treated successfully with 6 doses of rituximab, partial tumor resection, radiotherapy (20 Gy to both tonsils plus 10 Gy boost to the PTLD site) and DLI from the 2nd donor (infusions of 2 x 10^4 - 4 x 10^6 CD3+ cells/kg body weight). PTLD tissue from the biopsy and the partial resection were analysed for the origin of both the B- and the T-cells of the lesion. Chimerism analysis performed with short tandem repeat systems revealed no autologous signals; approximately 50% of the cells were from the first and second donor, respectively. XY-fluorescence-in-situ-hybridisation demonstrated a B cell PTLD originating from the first donor surrounded by T-cells from the second donor. The EBV load was excessively elevated at diagnosis of PTLD and reflected the course of the disease. The boy is in complete remission of PTLD with undetectable EBV load in his peripheral blood for 18 months now
This case is noteworthy and unique, because it demonstrated for the first time that EBV-infected B cells from a first donor can be the source of a PTLD after a second myeloablative BMT from a different donor. This B cell lymphoproliferation was under control by T cells from the second donor.

194 (RegID: 1243; 1244)
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DETAILED CHARACTERISATION OF EPSTEIN-BARR VIRUS (EBV) INFECTED CELLS IN THE CIRCULATION OF ORGAN TRANSPLANT RECIPIENTS WITH PERSISTENT HIGH EBV DNA LOADS.

AE Greijer, SJ Stevens, SA Verkuijlen, H Juwana, SC Fleig, EAM Verschuuren, BG Hepkema, JM Middeldorp

Posterabstract:

Post-transplant lymphoproliferative disease (PTLD) is a diverse group of mostly EBV-driven B-cell lymphomas occurring after solid organ or stem cell transplantation. In this study, solid organ transplant (SOT) patients (n=16) with chronic high levels of EBV without developing PTLD were analysed for EBV RNA profiles and the localization of the viral DNA in the circulation. In these SOT recipients, circulating EBV DNA load was always cell-associated, as plasma was EBV DNA-negative or low in presence of high loads in whole blood. FACS sorting of PBMC's revealed that EBV DNA was predominantly confined to the B-cell population. In two patients EBV DNA could be detected in the T cells, both CD4 and CD8, and in the monocytes. RNA patterns of these chronic active infected EBV patients showed only presence of the BARTs. Transcripts of LMP2a were weakly positive in 3 samples of which two had simultaneously LMP1 RNA expression. The expression of LMP1 and 2a were not related with high viral loads in these patients. RNA of EBNA1, LMP2b and ZEBRA remained negative, suggesting absence of cell division (EBNA1-) and lytic virus production (ZEBRA-) in the circulation of these patients.

In conclusion, chronic EBV infected SOT recipients have cell-associated EBV DNA, mainly located to B-cells. Our data indicate that EBV infected cells are generated and replicate outside the circulation and occasionally comprise leukocyte populations other than B-cells.

195 (RegID: 1274; 1275)

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EXPRESSION AND FUNCTION OF LFA-1 AND ICAM-1 IN NASAL NK/T CELL LYMPHOMA

Miki Takahara, Kan Kishibe, Shigetaka Moriai, Hideyuki Ishii, and Yasuaki Harabuchi.

Posterabstract:

Nasal NK/T-cell lymphoma is characterized by progressive necrotic lesions with infiltrative tumor cells in the nasal cavity. Prognosis of the lymphoma have very poor with progressive infiltration of lymphoma cells in local lesion and/or distant metastasis. Therefore, it is necessary for the treatment of the lymphoma to understand of the tumor characteristics. Previously we reported that the lymphoma cells are infected with Epstein-Barr virus (EBV), and that EBV is related to pathogenesis of the lymphoma through cytokines such as IL-9 and IL-10. However the characteristics were not fully understood.

It is known that cell adhesion molecules are related to progression of several kinds of malignancies. However, in hematopoietic malignancies, especially nasal NK/T-cell lymphoma, role of the molecules have not been examined in detail yet. LFA-1 (Leukocyte Function-associated Antigen-1) and its ligand, ICAM-1 (Intercellular Adhesion Molecule-1), which are typical cell adhesion molecules, take important role in attack of NK-cells to target cells. LFA-1 on NK-cells is combined with ICAM-1 on target cells, and signals from LFA-1 enhance activation and proliferation of NK-cells for elevation of offensive power of NK-cells. We examined the expression and function of the two molecules in EBV positive (SNK6) and EBV negative (KHYG-1) cell line. Moreover, because we previously reported existence of soluble ICAM-1 in serum form nasal NK/T-cell lymphoma, soluble ICAM-1 was also measured in culture supernatant of SNK6 cells.

Flow cytometric analysis revealed that both LFA-1 and ICAM-1 were expressed on both SNK6 and KHYG-1 cells. ELISA analysis revealed that soluble ICAM-1 was detected in culture supernatant of SNK6 cells, but not in supernatant of KHYG-1. Moreover, MTS assay showed that proliferation potency of the SNK6 cells decreased on culture in presence of LFA-1 blocking antibody. Therefore, combination of LFA-1 and ICAM-1 enhanced proliferation potency of SNK6 cells.

196 (RegID: 1525)
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DISSECTING THE ROLE OF SAP IN ANTI-EBV RESPONSES THROUGH EXAMINATION OF XLP CARRIERS

Palendira. U1, Hislop. A2, Rickinson. A2 and Tangye.S.G1

Posterabstract:

X-linked lymphoproliferative (XLP) disease is an inherited immunodeficiency characterised by extreme sensitivity to Epstein Barr Virus (EBV) infection. XLP patients are typically asymptomatic prior to EBV exposure. However, EBV induces the clinical disease in > 90% of affected individuals. In contrast to EBV infection of healthy individuals, which is self-limiting, exposure of XLP patients to EBV induces a vigorous and uncontrolled immune response involving polyclonally activated lymphocytes and monocytes. Despite such immune activation, XLP patients fail to control EBV infection resulting in severe and often fatal IM (58% of cases), hypogammaglobulinaemia (30% cases) and importantly B-cell lymphoma (35% of cases). A number of specific defects have been identified in lymphocytes from XLP patients. These include an impairment in cytotoxic function of CD8+ T cells and NK cells, abrogated development of NKT cells, a deficiency in memory B cells and impaired effector function of CD4+ T cells. The gene mutated in XLP, SH2D1A, encodes the cytoplasmic SH2-domain containing adaptor protein SAP (SLAM-associated protein) which associates with the cytoplasmic domains of members of the SLAM family of cell surface receptors. Due to the complications in XLP patients, it would be desirable to examine lymphocyte function in cells that are genetically identical except for the presence or absence of functional SH2D1A. We therefore have been studying female carriers of XLP. We have been able to detect SAP deficient and SAP sufficient CD8+ T cells in XLP carriers. Using this model, we have been able to identify phenotypic and functional differences between SAP+ and SAP- CD8+ T cells. We have also been characterizing the responses to not only EBV, but also Cytomegalovirus (CMV) and Influenza in these XLP carriers.

197 (RegID: 1723)
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THE OVERLAPPING TRANSCRIPTIONAL PROGRAMS OF LMP1 AND BLIMP1 SUPPORT A CRITICAL ROLE FOR LMP1 IN THE ABERRANT DIFFERENTIATION OF B CELLS.

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Posterabstract:
The latent membrane protein 1 (LMP1) of the Epstein Barr virus (EBV) is believed to be important for the transformation of germinal centre (GC) B cells which may contribute to the development of EBV associated lymphomas, such as Hodgkin lymphoma. When expressed in GC B cells, LMP1 induces transcriptional changes that show a striking overlap with those induced in the same cells by the B lymphocyte-induced maturation protein-1 (BLIMP1), a key transcription factor that is essential for plasma cell differentiation. For example, LMP1 and BLIMP1 coordinately regulate 230 genes, including the B cell differentiation-associated transcription factors, BCL6, PAX5 and IRF4. However, this mimicry is only partial, as unlike LMP1, BLIMP1 does not up-regulate the anti-apoptotic gene BCL2A1 or the chemokine CCL22. In addition, a proposed function of LMP1 is the up-regulation of the inhibitor of DNA binding 2 (ID2), which can inhibit PAX5 mediated maintenance of B cell identity. However, ID2 is not regulated by BLIMP1. Furthermore, the similarity between LMP1 and BLIMP1 targets is not a simple consequence of BLIMP1 up-regulation by LMP1. Additionally, LMP1 down-regulates BLIMP1 in GC B cells. Our data suggest that while LMP1-expressing cells are driven into the post-GC stages of B cell differentiation, they fail to induce BLIMP1 and so are prevented from completing plasma cell differentiation. Given that plasma cell differentiation is associated with induction of the virus replicative cycle, then the LMP1-mediated disruption of this process could facilitate viral persistence.

198 (RegID: 1731)

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LACK OF FUNCTIONAL SAP MAY LEAD TO EVASION OF APOPTOSIS, A CLUE TO THE CLINICAL PICTURE OF XLP

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Posterabstract:

Deletion or mutation of the SAP gene is associated with the X-linked lymphoproliferative disease (XLP), a primary immunodeficiency characterized by unusually serious, often fatal infectious mononucleosis and by a 200 times higher risk for lymphoma development than the general population. SAP functions have been linked to signal transduction pathways that control T and NK cell activation/ function. However, our results show that SAP is involved in other cellular functions as well.

Upregulation of SAP in the late phases of T cell activation suggests a role in T cell homeostasis. In experiments using clones of the T-ALL line CCRF-HSB2 differing in SAP expression levels, we obtained evidence that SAP is instrumental in apoptosis. Clones with higher levels of SAP were more prompt to activation induced cell death (AICD), manifested by caspase activation, PARP cleavage.

Our previous finding that p53 induces SAP expression motivated us to study the impact of SAP in DNA damaged cells. We found that when SAP was introduced in lymphoblastoid cell lines (LCL) derived from XLP patients, DNA damage induced significantly higher number of dead cells compared to the original LCLs that lack SAP.

In an attempt to identify the mechanism of how SAP regulates apoptosis sensitivity, we have performed immunoprecipitation experiments and found that SAP binds to the anti-apoptotic protein VCP (valosin containing protein). It is conceivable that by binding VCP, SAP can prevent it from exerting its anti-apoptotic function, thus a net result being a more pro-apoptotic phenotype of the cells that express SAP.

Our results suggest that through its involvement in the apoptotic pathways, SAP contributes to the maintenance of T cell homeostasis and to the elimination of potentially dangerous DNA damaged cells. This new, pro-apoptotic, function of SAP would explain why XLP patients exhibit uncontrolled T cell proliferation in fatal IM and develop lymphomas with high frequency.

199 (RegID: 1803)
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INDUCTION OF EFFICIENT DIFFERENTIATION AND SURVIVAL OF PORCINE NEONATAL PANCREATIC CELL CLUSTERS USING AN EBV-BASED PLASMID EXPRESSING HGF

Min Sun Kim, Ji-Won Kim, Chenglin Sun, Sang Taek Oh, Kun Ho Yoon and Suk Kyeong Lee

Posterabstract:

Porcine neonatal pancreatic cell clusters (NPCCs) have been actively studied as a source of pancreatic stem cell transplantation for the treatment of diabetes. In this study, the hepatocyte growth factor (HGF) gene was cloned in an EBV-based plasmid vector (pEBVHGF) and the effects of the HGF expression on the survival and differentiation of NPCCs were analyzed. For comparison, pHGF was constructed by deleting EBNA-1 and OriP from pEBVHGF. The expression of HGF, as measured by ELISA, lasted longer when pEBVHGF was used than when pHGF was used. C-Met phosphorylation co-related with the expression of HGF in the transfected NPCCs. Immunocytochemistry experiments showed that NPCCs showed a higher and longer expression of insulin when they were transfected with pEBVHGF than with pHGF. Moreover, a greater number of NPCCs survived for a longer period after they were transfected with pEBVHGF than when they were transfected with pHGF. Taken together, these results indicate that transfecting NPCCs with the HGF gene using an EBV-based plasmid is a more effective method of inducing differentiation to beta cells and enhancing survival than using a conventional plasmid. Therefore, it may be possible to use EBV-based plasmids to modify pancreatic stem cells for xenotransplantation.

200 (RegID: 1832)

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DIFFERENTIALLY EXPRESSED HOST GENES IN RELATION TO VIRAL LOADS IN EPSTEIN-BARR (EBV) VIRUS INFECTION AFTER PEDIATRIC ORGAN TRANSPLANTATION

Upton Allen, Michelle Barton, Joseph Beyene, Pingzhao Hu, Nasser Khodai-Booran, Diane Hébert, Anne Dipchand, Vicky Ng, Melinda Solomon, David Grant, Bo Ngan, Maria Zielenska, and Sheila Weitzman. Hospital for Sick Children, Toronto, Canada.

Posterabstract:

INTRODUCTION: There is an inconsistent relationship between elevated EBV loads and the risk of progression to post-transplant lymphoproliferative disorder (PTLD). We hypothesized that aspects of the virus-host interaction could be measured to help predict progression to PTLD.

OBJECTIVE: To examine differentially expressed host genes and their interaction patterns in different EBV viral load settings after transplantation compared with healthy controls.

METHODS: Gene expression was measured by microarray analysis of RNA from CD19+ B lymphocytes using the Human Genome U133 plus 2.0 GeneChip. Moderated t-statistics were computed for each gene using a local pooled error (LPE) test. The false discovery rates (FDR) of the differentially expressed genes were evaluated and validated using RT-PCR. Using average-linkage hierarchical clustering analysis, a set of genes with the largest variation, as measured by coefficient of variation, was used to explore the patterns of viral loads (VL). Gene network analysis was also performed.

RESULTS: Among 27 samples from 26 transplant recipients, there were 4 episodes of PTLD. The VL categories were: low or undetectable loads (LU), N = 14; high or intermediate loads (HI), N = 13. There were 7 healthy EBV-seropositive (P) and -seronegative controls (N). Median age of transplant patients = 12.1 yrs (range 1-16.9); Median time post-transplant 0.5 yr (range 0.1-3.8). Using the LPE test, we identified 24-54 differentially expressed genes with FDR<=0.1 in each of four comparisons of HI vs P, LU vs P, HI vs LU and P vs N, respectively. Clustering analysis of 563 gene expressions identified 5 clusters aligned with levels of VL. PTLD occurred in 4 of 5 clusters. Modules with interacting genes were examined.

CONCLUSIONS: These data revealed a spectrum of genes that includes several relevant to the pathogenesis of PTLD. These include genes involved in modulating the TH1/TH2 axis in the setting of EBV infection.

201 (RegID: 1863)
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CASE REPORT OF EBV POSITIVE CLASSICAL HODGKIN LYMPHOMA IN AN ADULT WITH POLYCYSTIC KIDNEY DISEASE 15 YEARS PO

Bacani JT (UofA Hospital), Sis B(UofA Hospital), Slack GW (Cross Cancer Institute) and Ingham RJ (UVic)

Posterabstract:

Post-transplant lymphoproliferative disorders (PTLD) are recognized as a complication of immunosuppression and occur with a 1-8% reported incidence in solid organ transplantation recipients. PTLD are classified into two major categories, polymorphic and monomorphic PTLD. The majority of the monomorphic PTLD are non-Hodgkin's lymphoma (NHL) of B-cell origin. Hodgkin lymphoma (HL) is not part of the typical spectrum of PTLD and thus rarely reported in this population as a de novo malignancy. However, HL is recognized as part of the disease spectrum of PTLD. Epstein-Barr virus (EBV) has been implicated in both post-transplant NHL and HL. Rarer still, specifically in the context of polycystic kidney disease (PKD) which account for 10-15% of end stage renal patients ultimately requiring renal transplantation, is the development of HL. Here we report the first adult case report of an EBV-positive classical HL in an adult male with PKD. A 55-year-old man following renal transplantation 15 years ago for PKD presented with abdominal distension, B symptoms, and bipedal edema. He was also anemic and leukopenic. Imaging showed polycystic liver and kidney, splenomegaly and significant lymphadenopathy. A biopsy of a mobile right cervical posterior triangle lymph node showed classical HL with readily evident classical Reed-Sternberg (RS) cells which strongly express CD15, CD30, PAX-5, EBER and do not express CD45, CD20, ALK-1. Preferential kappa and lambda light chains expression was not evident. Scattered reactive CD3 positive T-cells were also present. No other site of HL was identified. He initially received a short course of valgancyclovir and subsequently underwent chemotherapy (adriamycin, bleomycin, vinblastine and decarbazine). Follow-up 9 weeks after initial admission and 7 weeks after chemotherapy resulted in resolution of most of his presenting symptoms and complete resolution of mediastinal lymphadenopathy on imaging.

NOTES:

Poster sessions

Session 9: Diagnostics

202 (RegID: 1221; 1222)

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AN ALTERNATIVE FLUORESCENCE METHOD TO DETECT ANTIBODY AGAINST EPSTEIN-BARR VIRUS DNASE, A USEFUL SEROMARKER IN SCREENING PATIENTS WITH NASOPHARYNGEAL CARCINOMA

Sheng-Ping Chou, Mei-Ying Liu, Jen-Yang Chen

Posterabstract:

Human antibody which can neutralize Epstein-Barr virus (EBV) DNase activity has proven a useful seromarker in screening patients with nasopharyngeal carcinoma (NPC). A conventional way to detect the neutralizing antibody was done by DNase neutralization assay in which the DNA substrate needed to be radiolabelled by 14C for sensitivity. However, this results in inconvenience in material preparation, increase in isotope contamination risk and money cost. A better way to overcome these drawbacks was created by applying PicoGreen fluorescence in the DNase neutralization assay. Optimal condition for the PicoGreen-assay was set step by step and tested for 186 human sera which were also analyzed in parallel by the 14C-assay. Comparing data from these two methods by Kappa Statistics, a Kappa coefficient of 0.818 was obtained and it indicates that the new PicoGreen-assay shows almost perfect in agreement with the old 14C-assay. It is concluded that the easier, saver and cheaper PicoGreen-assay can replace the traditional 14C-assay to detect antibody neutralizing EBV DNase activity and will be more applicable in screening NPC patients.

203 (RegID: 1291)

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EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN -1 (LMP1) EXPRESSION IN TWO AGE GROUPS OF NASOPHARINGEAL CARCINOMA PATIENTS STRATIFIED FOR TNM-STAGE.

Ikasari Muhtadi, Marlinda Adham,Armiyanto, Averdi Roezin, Ria Kodariah, AN Kurniawan, Rianto Setiabudy and Jaap M.Middeldorp

Posterabstract:

At the ENT-oncology outpatient clinic at Dr. Cipto Mangunkusumo Hospital, Nasopharyngeal Carcinoma (NPC) before age of 30 years was 12.89% of all NPC patients in 2000, increasing to 23,53% in 2005. Many differences in biological profile between young and adult NPC were found.

Objectives: This research aims to define the difference of EBV-LMP1 expression in NPC, between patients <30 years old and ≥30 years old in Indonesia. Moreover, we aim to define the relationship between EBV-LMP1 expression in NPC with sex, histopathological type and the stage of the disease.

Methods: Twenty-five EBER-RISH confirmed NPC WHO-III cases from each age group, stratified for having same TNM-stage and gender, were included. The stage of LMP1 expression was compared pairwise for age with matching of sex, by using immunohistochemistry staining with anti-LMP1 OT21C monoclonal antibody. Score was calculated semi-quantitatively from 500 cells in 5 different areas at 400x.

Results: LMP1 expression was found in 75% cases with a score between 0.2 to 11.7 (Khabir et al., Virol J. 2005; 2:39). The average score for patients <30 years was higher, but was not different significantly ($p>0.05$). LMP1 expression was not associated with sex, histopathology findings and the stage of disease. LMP1 levels had borderline significance for tumor size, but it didn't have relationship with neck node and metastatic status.

Discussion: The overall score of EBV-LMP1 expression in this study was similar or higher compared to other studies, relating to factors as race, immune status, food components and method and materials used. Higher LMP1 expression in patients <30 years old confirms findings by others, and suggests more aggressive tumor behavior with loco-regional tumor progression at young age.

It is suggested that NPC pathogenesis at young age is influenced by LMP1.

204 (RegID: 1291)
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NASOPHARYNGEAL BRUSH PAP-SMEAR CYTOLOGY AS SIMPLE YET EFFECTIVE METHOD FOR DETECTION OF NASOPHARYNGEAL CARCINOMA (NPC) IN DEVELOPING COUNTRIES.

Lisnawati , Ria Kodariah, Linda Adham , Kurniawan, Jaap M.Middeldorp

Posterabstract:

NPC is a leading cancer in many parts of SE-Asia and nearly 100% EBV-associated, allowing use of EBV-based diagnostic techniques. Early-stage detection is desired but difficult due to non-specific symptoms. Simple and cheap diagnostic methods are needed allowing early-stage detection. Current diagnosis needs an invasive biopsy and pathological examination, preferably with detection of EBER-RNA. Recently we described a non-invasive brush technique with EBV-DNA load detection which may replace the biopsy (Stevens et al., Int. J. Cancer (2006), 119 (3): 608-14).

Purpose: In this study we evaluate the use of “brush cytology” on 38 routine nasopharyngeal (NP) brushings, collected at ENT-department of Dr. Cipto Hospital in Jakarta. From each NP brush 4-6 slides were smeared and the remainder was placed in Lysis buffer for EBV-DNA load measurement using LightCycler PCR.

For tumor cell detection we used Giemsa or quick-PAP staining, EBER-RISH, LMP1-IHC. In all cases a biopsy was taken from the same site as brush and analysed by standard histochemistry, EBER-RISH (Dako) and LMP1-IHC (OT21C MoAb).

In 33/38 subsequent cases with suspected NPC, biopsy revealed EBER+ tumor cells with WHO-III classification in 30/33 cases.

In 32/33 cases groups of tumor cells were visible by routine PAP staining whereas non-NPC cases were all negative for tumor cells in the cytosmear.

EBER-RISH on alcohol fixed smears yielded a clear positive reaction in 19/20 cases analysed and LMP1-IHC yielded moderate-strong cytoplasmic/membrane staining in 26/30 cases. In all NPC cases significant EBV-DNA load was detected in the remainders of the cytobrush after repeated glass smearing.

These data show that simple NP-brushing combined with routine cytological examination is feasible for detection of NPC in routine ENT-wards in developing countries and may prove equally effective as cytological detection of cervical cancer.

205 (RegID: 1292)

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EBV-DNA LOAD MONITORING IN NASOPHARYNGEAL (NP) BRUSHINGS AND WHOLE BLOOD DURING AND AFTER THERAPY OF NASOPHARYNGEAL CARCINOMA (NPC).

Linda Adham, Alida Harahap, I.Bing Tan, Sabine C Fleig, Astrid E Greijer and Jaap M Middeldorp.

Posterabstract:

NPC is a leading cancer in SE-Asia and nearly 100% EBV-associated, allowing use of EBV-based diagnostic techniques. Recently we described a non-invasive NP-brush and whole blood approach combined with EBV-DNA load measurement for the diagnosis of NPC (Stevens, 2005, 2006), yielding better sensitivities in NP-brushings.

Purpose: In this study we evaluate the use EBV-DNA load monitoring in consecutive whole blood samples and NP brushings taken at diagnosis and defined time points during and after chemoradiation therapy in a series of 235 NPC patients. EBV-IgA serology was also done at all timepoints and routine clinical examinations, CT-scan, nasendoscopy and biopsy were performed on a regular basis during follow-up.

Results: In all confirmed NPC cases, EBV-DNA load in NP-brushings at diagnosis showed strongly elevated levels in nearly 98% of patients, with sometimes extreme high levels (>50x106c/brush), whereas EBV-DNA was detectable in parallel blood samples mostly at low levels, being above the 2000c/ml cut-off in only 40% of cases.
During therapy rapid and significant declines (>1000-fold) of EBV-DNA load in NP-brushings associated with good clinical responses, whereas cases with partial response and persistent disease showed stable or rising EBV-DNA levels. In patients without whole blood EBV-DNA load at diagnosis, fluctuating positive levels were detected during therapy, but varied strongly per patient. Patients with detectable EBV-DNA loads before therapy maintained fluctuating levels during and post- therapy.
EBV-IgA serology was not predictive of therapy outcome at short term but may be useful to (cheaply) monitor for disease relapse at longer intervals post-therapy.

Conclusions: Our results indicate that EBV-DNA load monitoring in NP-brushings provides highly relevant data reflecting tumor behaviour in situ and can be used for assessment of treatment effect. EBV-DNA in blood is less informative, more variable and was useful in only a limited number of patients. EBV-DNA in blood possibly reflects the apoptosis sensitivity/activity of the tumor, showing significant individual variation. The non-invasive NP-brush technique allows repeated sampling without much discomfort to the patient, and can replace the invasive biopsy, while providing more relevant information on presence and activity EBV+ NPC in situ.

206 (RegID: 1293)
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RAPID TEST FOR DIAGNOSIS OF MONONUCLEOSIS AND EBV CARRIERSHIP BASED ON EBV-SPECIFIC PEPTIDES AND MONOCLONAL ANTIBODIES DETECTING IGM-VCA- AND IGG-VCA/EBNA1/EAD

JM Middeldorp, S Stevens, SAMW Verkuijlen, D Schol and R van Herwijnen

Posterabstract:
Purpose: Acute and active EBV infection is accompanied by a variety of “non-specific” symptoms resulting from associated inflammatory responses. Similar symptoms are observed in other acute and chronic diseases requiring specific laboratory testing. Diagnosis of EBV infection demands the use of multiple tests for the detection of IgM and IgG antibodies to various EBV antigens, which complicates direct test options (bed side or doctor’s office).
Approach: Using IgM- and IgG-specific monoclonal antibodies and combinations defined biotinylated synthetic peptides or purified recombinant proteins representing immuno-dominant epitopes of EBV VCA, EBNA1 and EAd complexes linked to nitrocellulose membranes (capture) and 10-20nm gold particles (detection signal), we were able to construct simple membrane-based “rapid tests” for the specific detection of EBV-reactive IgM-VCA or IgG-VCA/-EBNA1/-EAd in human sera with simple handling and 10-15 min. reaction time.
Results: More than 50 sera from mononucleosis (IM) patients were correctly diagnosed by a positive IgM-VCA and negative EBNA1-IgG reaction. Follow-up samples showed appropriate IgM/IgG-VCA dynamics, correlating well with IgM-/IgG-ELISA values used as standard. EAd-IgG was temporarily found in IM patients but not in healthy EBV carriers. Sera from 25 patients with various acute non-EBV viral infections and autoimmune diseases gave negative EBV-IgM results. RF-positive sera gave no non-specific results. Sera from healthy EBV carriers were identified by a positive reaction for IgG-VCA/EBNA1, with a negative response for EAd-IgG. Photometric scanning of the marker bands in situ, including the positive control, allowed for semi-quantification.
Conclusion: This new approach allows complex EBV diagnostic serology using multiple distinct antigen-antibody markers to be used in an accurate single rapid test format, permitting direct application in the doctor’s office.

207 (RegID: 1526)
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EVALUATION OF 3 NEW VIDAS TESTS FOR THE DETECTION OF EPSTEIN-BARR VIRUS VCA IGM, VCA/EA IGG AND EBNA IGG ANTIBODIES IN HUMAN SERUM SAMPLES

M. Baccard, A. Foussadier, L. Allard, P. Desmottes, P. Morand, J.M. Seigneurin

Posterabstract:

Objectives:

The aim of the study was to evaluate the performance of 3 new automated VIDAS EBV diagnostic tests for the determination of the patient EBV serological status. The EBV reagents currently under development on VIDAS instrument are aimed to detect immunoglobulins against Viral Capsid Antigen (VCA), Early Antigen (EA) and Epstein-Barr nuclear antigen (EBNA) : VIDAS VCA IgM, VIDAS VCA/EA IgG and VIDAS EBNA IgG. Sensitivities and specificities were determined on characterized sera samples for each of the 3 tests.

Methods:

VIDAS EBV VCA/EA IgG and EBNA IgG tests are based on indirect sandwich format, with peptides coated on the solid phase. VIDAS EBV VCA IgM test is based on immunocapture with labelled peptides as revelation phase.

Fifty seven serum samples from EBV negative individuals, 109 from EBV primary infection and 93 from EBV past infection were tested on VIDAS. EBV clinical status was initially established using 2 microtiter plates tests: Dade Behring, which detects EBV IgM and EBV IgG, and BMD for the specific detection of EBNA IgG. Discrepant results were analysed using IF.

Results:

On characterized serum samples, the sensitivities of VIDAS VCA IgM, VCA/EA IgG and EBNA IgG were 97.5 % [93.6 – 99.0%], 95.9 % [92.0 –97.9%] and 98.1 % [93.2- 99.5%] respectively and the specificities of VIDAS VCA IgM , VCA/EA IgG and EBNA IgG were 95.2 % [88.2 – 98.2%], 100 % [93.4 – 100%] and 96.5 % [91.9 – 98.5%] respectively.

Conclusions:

The new VIDAS EBV reagents presented good performance in accordance with the patients' serological status established with the other immunoassay methods. VIDAS EBV reagents will be a good alternative to currently used manual method for Infectious Mononucleosis determination, enabling single testing as well as series.

208 (RegID: 1786; 1788; 1792)

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MCP-1 PROMOTER POLYMORPHISM AT -2518 IS ASSOCIATED WITH METASTASIS OF NASOPHARYNGEAL CARCINOMA AFTER TREATMENT

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Affiliations: [1]Institute of Microbiology and Immunology, National Yang-Ming University, Taipei; [3]Chang Gung Molecular Medicine Research Center and Departments of [4]Life Science and [8]Public Health, Chang Gung University, Taoyuan; Departments of [2]Radiation Oncology ,[5]Medical Research, [6]Pathology, and [7]Otolaryngology, Chang Gung Memorial Hospital at Lin-Kou, Taoyuan, Taiwan.

Posterabstract:

Nasopharyngeal Carcinoma (NPC) is a unique tumor with a restricted geographical and population distribution, and is notorious for its highly metastatic potential. In Taiwan, around 20 - 25% of NPC patients develop distant metastasis after initial therapy. Previous literatures suggested that the risk of NPC occurrence and patient's responses to therapies are closely associated with genetic background, however, only susceptible genes were found. Monocyte Chemoattractant Protein 1 (MCP-1) is a potent chemoattractant protein for macrophages and T cells, which are the major cell types infiltrated in NPC tumor mass. The expression of MCP-1 in response to various stimuli has been reported to be modulated by a functional single nucleotide polymorphism (SNP-2518) located in the distal regulatory region of *MCP-1* gene. We retrospectively analyzed *MCP-1* SNP-2518 genotypes and their association with clinical outcomes in 411 NPC patient's genomic DNA, collected over 10 years, in Chang Gung Memorial Hospital. Our study demonstrated that *MCP-1* SNP-2518 genotype is closely associated with the risk of developing distant metastasis after complete treatment. According to the immunohistochemical data, we found that the MCP-1 protein is overexpressed in more than 80% NPC biopsies. Moreover, the MCP-1 protein expression levels in NPC tumor cells are correlated with the *MCP-1* SNP-2518 genotype and the intensities of macrophages infiltrated in the tumor mass. All these data implying that the expression level of MCP-1 can be modulated by the functional SNP, and result in varied amount of macrophage be recruited to the tumor mass. This is the first report suggesting that a host genetic polymorphism that may serve as a prognostic predictor for NPC and may help to develop personalized medicine after validation.

NOTES:

Poster sessions

Session 10: Vaccine and Immunotherapy

209 (RegID: 1065)
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EBV-SPECIFIC T CELL-BASED IMMUNOTHERAPY FOR NASOPHARYNGEAL CARCINOMA

Corey Smith, Leone Beagley, Leanne Cooper, John Nicholls, Daniel Chua and Rajiv Khanna

Posterabstract：
Epstein Barr Virus (EBV) is associated with a number of malignancies including Hodgkin's Lymphoma (HL) and Nasopharyngeal carcinoma (NPC) that can arise in both immunocompetent and immunocompromised individuals, including HIV-infected individuals. Although successful in the majority of cases, current therapies are less effective in patients who relapse or are at later stages of disease and can be associated with complications in immunosuppressed patients. Vaccination or immunotherapeutic treatment with cytotoxic T lymphocytes (CTL) offers an alternative therapy for the treatment of NPC. Recent success in treating Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (PTLD) using cytotoxic T cell (CTL) based immunotherapy has led to interest in the development of CTL based immunotherapy to treat other EBV-associated malignancies, including NPC. However, unlike PTLD which typically express the full array of EBV latent antigens, NPC cells express a limited array of antigens, including the latent membrane proteins (LMP) 1 and 2, and EBV nuclear antigen (EBNA) 1. These antigens are typically poorly immunogenic, evolving to limit recognition by the immune system, resulting in the induction of sub-dominant T cell responses. Therefore an immunotherapeutic approach for the treatment of NPC will need to be designed to specifically target these antigens. We have developed a novel antigen presentation system based on a replication-deficient adenovirus which encodes multiple HLA class I-restricted LMP1 and LMP2 epitopes covalently linked to the EBNA1 protein (referred to as AdE1-LMPpoly). We have demonstrated in healthy individuals and cancer patients, both NPC and HL, that a single stimulation with AdE1-LMPpoly can be employed to rapidly expand T cells specific for all three antigens, inducing a higher specificity of T cells than that generated using EBV-transformed lymphoblastoid cell lines, the conventional strategy for the production of T cells to treat EBV-associated malignancies. This strategy has is currently being trialled in stage IV NPC patients.

210 (RegID: 1137)

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EBV ANTIGEN AND IMMUNE RELATED PROTEINS EXPRESSION ASSOCIATED WITH THE IMMUNE REGULATION IN NASOPHARYNGEAL CARCINOMA (NPC) PATIENTS: IMPACTIONS FOR EBV-SPECIFIC CTL ADOPTIVE IMMUNOTHERAPY

Jiang Li1,2,3, Hao-yuan Mo4, Fang Qiu4, Yi-Nan Zhang1,2,3, Qiu-Yan Chen4, Yong-qiang Li1,2,3, Li-Xi Huang1,2,3, Maria G Masucci5# and Yi-Xin Zeng1,2,3#

Posterabstract：

Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV) associated malignancy with high prevalence in Southern Chinese. Adoptive immunotherapy with EBV-specific CTLs (EBV-CTL) has been used to treat EBV-associated nasopharyngeal carcinoma (NPC) but only a fraction of the patients shows noticeable clinical response. In order to assess whether defects of EBV-specific immunity may contribute to the tumor and affect the efficiency of EBV-specific CTL adoptive immunotherapy, the phenotype and function of circulating T-cells were investigated in untreated NPC patients. Here we show that NPC patients can be divided into two groups based on the percentage of CD3+ T cells in peripheral blood at the time of first admission to the clinic, (>52.6%, mean-2SE from healthy controls, NPC Group I; <52.6%, NPC Group II). The patients in Group II show a significantly decrease of CD3+CD8+, CD3+CD4+ and CD3+CD45RO+ memory T cells, and increase of CD3-CD16+ NK cells compared to Group I patients and healthy controls (P<0.001). EBV-specific T cell responses, as assesses by the generation of EBV-CTL lines and production of IFN- . upon stimulation with autologous EBV infected cells are also weaker in this group of patients.

Furthermore, To understand the role of Epstein-Barr virus (EBV) and viral products in associated with immunophenotype and clinical outcome of primary nasopharyngeal carcinoma (NPC), the expression levels of chemokines IFN-γ-induced protein 10 (IP-10, CXCL10), stromal-derived factor-1 (SDF-1, CXCL12) and its receptor CXCR4 was investigated in primary NPC biopsy specimens from these two groups NPC patients in parallels with LMP1 antigen and EBER1 by immunohistochemisty (IHC) and in situ hybridization (ISH). The tumors express low levels of the EBV encoded latent membrane protein (LMP)-1 and HLA class II (P<0.05) in NPC Group II patients compared with NPC Group I, and the expression levels of others proteins in tumors were no significant difference in these two groups NPC patients. In addition, Our results showed that (a) the elevated expression levels of IP10, SDF-1, CXCR4, -microglobulin, HLA-DR, and CD54 in NPC lesions was 66%, 36%, 30%, 42%, 55% and 69%, respectively. (b) High expression of SDF-1 was observed in advanced NPC (N stage, P<0.05). (c) The tumors express low levels of the EBV encoded latent membrane protein (LMP)-1 and HLA class II (P<0.05) in NPC Group II patients compared to NPC Group I patients.

Taken together, our findings provide new insights on the molecular characteristic of the malignant cells which may affect the outcome of immunotherapy in NPC; and these finding demonstrate that NPC patients may be distinguished on the basis of their EBV specific immune status may explain why some patients respond poorly to EBV-CTL immunotherapy.

211 (RegID: 1141)

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VACCINATION OF PATIENTS WITH EPSTEIN-BARR VIRUS (EBV) POSITIVE MALIGNANCY WITH MODIFIED VACCINIA ANKARA EXPRESSING A CHIMAERIC EBV EBNA 1 AND LMP2 ANTIGEN

Graham Taylor 1, Edwin Hui 2, Anthony Chan 2, Kevin Harrington 3, Alan Rickinson 1, Neil Steven 1

Posterabstract

Epstein-Barr virus is present within the malignant cells of several human cancers including almost all undifferentiated nasopharyngeal carcinoma (NPC) and 40% Hodgkin's lymphoma. In malignant cells, viral latent antigen expression is restricted to EBNA1, LMP2 and LMP1 whereas immunodominant targets for MHC class I restricted T cell responses are down-regulated. The EL vaccine encodes a fusion gene comprising EBNA1 c terminal 363-641 spliced to complete LMP2. This includes multiple MHC class I restricted epitopes in LMP2 known to be recognised by low abundance circulating cytotoxic T lymphocytes in some healthy donors and cancer patients, and also several MHC class II restricted epitopes in EBNA1.

To determine immunogenicity, a UK-based trial is recruiting patients with any EBV+ cancer outside the transplant setting, following one program of primary chemotherapy or chemoradiotherapy, in remission or for whom no other standard therapy exists. In parallel, in China a trial with the same design is recruiting NPC patients following first line therapy. These trials escalate from 5x10^7 to 5x10^8 pfu virus given intradermally three times at three week intervals. Seven and ten patients have been treated in the UK and China respectively and dosage has so far reached 3.3x10^8 pfu. The main toxicities have been injection site reactions and systemic 'flu like symptoms. Immune responses characterised to date indicate amplification of CD8+ T cell responses to LMP2 as well as CD8+ and CD4+ T cell responses to EBNA1 in a high proportion of vaccinated patients.

212 (RegID: 1455)
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TREATMENT OF EPSTEIN BARR VIRUS POSITIVE NASOPHARYNGEAL CARCINOMA WITH ADOPTIVELY TRANSFERRED CYTOTOXIC T LYMPHOCYTES

Louis CU, Straathof K, Gerken C, Cooper-Havlik D, Torrano V, Lopez T, Bollard CM, Gresik MV, Weiss H, Gee A, Brenner MK, Rooney CM, Heslop HE, Gottschalk S

Posterabstract:

Background:

Epstein-Barr virus (EBV)-specific cytotoxic T cells (EBV-CTL) are an attractive therapeutic option for nasopharyngeal carcinoma (NPC). We have conducted two Phase I clinical trials with EBV-CTL. In the first trial, EBV-CTL were given alone and in the second trial, we aimed to enhance in vivo expansion of EBV-CTL by lymphodepleting patients prior to CTL infusion. For lymphodepletion we used CD45 monoclonal antibodes (MAbs).

Study design:

The primary objective of these clinical trials was to determine the safety of escalating doses of EBV-CTL with or without lymphodepletion in EBV-positive NPC patients. The secondary objective was to determine the expansion, persistence and anti-tumor effects of infused EBV-CTL.

Results:

32 patients with advanced-stage NPC received EBV-CTL without dose-limiting complications. Prior to CTL infusion, 8 patients were in remission, 22 had active disease, and 2 had abnormal imaging studies of unknown significance. 7/8 patients in remission remain in remission 6-64 months post CTL. For the remaining 24 patients, the best overall response rate was 50% with 6 complete responses, 2 partial responses, and 4 with stable disease during a median follow-up of 9 mths. Of the 6 with a CR, 4 have been sustained 2-4 years, and 2 relapsed >2 years post CTL. 8 patients received CD45 MAbs prior to EBV-CTL and were evaluable for immune reconstitution analysis. Infusion of CD45 MAbs resulted in transient lymphopenia (resolved within 7 days), increased serum IL-15 levels in 6 patients, and significant expansion of EBV-CTL within 8 weeks post-infusion in 3 patients.

Conclusion:

Treatment of EBV-positive NPC with EBV-CTL appears to be safe and can be associated with significant anti-tumor activity. Lymphodepletion with CD45 MAbs prior to CTL infusion is safe and results in expansion of adoptively transferred CTL in a subset of patients. These encouraging results warrant further exploration of EBV-targeted immunotherapies for NPC.

213 (RegID: 1685; 1687)

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DEVELOPMENT OF ANTIBODY-BASED IMMUNOTHERAPY OF EBV ASSOCIATED MALIGNANCIES

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Posterabstract:

Purpose: The use of latent membrane proteins LMP1 and LMP2 as potential targets for antibody-based immunotherapy in Epstein-Barr virus (EBV) related malignancies was examined. LMP1 and LMP2 contain 6 and 12 transmembrane domains, respectively, linking the intracellular N- and C- termini and containing putative extracellular loops of variable length. Both proteins are poorly-immunogenic in vivo. The extracellular accessibility of LMP1 and LMP2 loops remains to be defined and is subject of this study.

Experimental design: To achieve extracellular targeting of LMP1 and LMP2, rabbit antibodies were developed against linear and circular peptides mimicking the extracellular loops of both proteins. The accessibility of LMP1 and LMP2 at the exterior of cells was explored on Ficoll-purified viable cells incubated on ice using FACS and confocal microscopy. To investigate the biosynthesis of LMP1 and LMP2 expression in the cell cycle, cells were synchronized using L-mimosine (G0/G1 and S phase) or demecolcine (G2/M phase).

Results: Using antibodies generated with circular peptides a clear staining of LMP1 and LMP2 on viable RAJI and X50/7 was observed. LMP1 and LMP2 both showed a heterogeneous distribution in plasma membranes in a small part of the population. Only 10% of RAJI cells were LMP1 positive and 7% of X50/7 cells were LMP2 positive. Synchronized cells showed different LMP1 and LMP2 localization patterns as compared to the untreated cells: less than 3 % of RAJI cells in the G0/G1 phase exposed LMP1 in the plasma membrane whereas 30 - 40% of the cells in the S and G2/M stage were LMP1 positive.

Conclusions: LMP1 and LMP2 have promising characteristics to serve as targets for antibody based therapy. LMP1 and LMP2 appear to localize in the plasma membrane in a cell cycle dependent way.

214 (RegID: 1869; 1870)
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DYNAMIC ANALYSIS OF EPSTEIN-BARR VIRUS IN THE PROCESS OF NASOPHARYNGEAL CARCINOMA THERAPY

Tang Faqing, Weiwei Wang, Ling Zhang, Lifang Yang, Meng Jingjing，Chunlei Xie, Yuping Liao, Ya Cao

Posterabstract:

Epstein-barr virus (EBV) is etiologically with nasopharyngeal carcinoma (NPC). An analysis of EBV DNA has been shown to be clinically useful for the early detection, monitoring and prognostication of NPC. EBV DNA levels appear to correlate with treatment response and they may predict disease recurrence, suggesting that they may be an independent indicator of prognosis. In the present, we dynamic detected EBV DNA copies of patients with NPC in radiotherapy process, and investigate the relationship of EBV copies and tumor sizes. EBV DNA in NPC patients' plasma was detected at one, two, three, four, five, six, seven weeks and three months after treatment using fluorescent quantitative nested-PCR technique and NPC tumor sizes were measured using magnetic resonance imaging (MRI) technology. EBV DNA was detected in 27/28 NPC patients plasma, and positive rate was 96.4%. The median concentration was 1800 copies/μl (range: 0-22100). Among 30 cases, EBV DNA were declining from 4.68´104 to 0, and MRI data showed that NPC tumor sizes decreased from 650mm3 to 0 after treatment. By analyzing relationships between EBV DNA copies and tumor size, we found that EBV DNA was closely associated with tumor volume to a certain extent. [This work is supported by the national science and technology 863 of China (Grant NO: 2006AA02Z481) and Program for New Century Excellent Talents in University (Grant NO: NCET-06-0685); National Natural Science Foundation of China (30572455)].

215 (RegID: 1923)

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GENERATING ANTI-LMP1 ANTIBODIES USING PEPTIDES ENCODING CONFORMATIONAL EPITOPES REPRESENTATIVE OF TWO ADJACENT EXTERNAL LOOPS ("INTER-LOOP" EPITOPES)

Claire Gourzones, Clément Barjon, Anne-Sophie Jimenez, Catherine Durieu, Catherine Delbende, Pierre Busson, Denis Tranchand-Bunel - CNRS UMR 8126 – Université Paris-sud – Institut de Cancérologie Gustave Roussy –94805 Villejuif Cedex – France (Claire.gourzones@igr.fr)

Posterabstract:

Administration of antibodies able to selectively bind targets at the surface of tumor cells has become a major therapeutic strategy against human malignancies. In EBV-associated maligancies, antibodies reacting with viral targets at the surface of tumor cells would be an interesting way to implement this strategy. Yet, in latently EBV-infected tumor cells, there are no viral proteins trafficking in the plasma membrane or only type III membrane proteins – LMP1 and LMP2 - which have no extra-cellular components except very short external loops. These external loops are poorly immunogenic and do not seem to elicit natural antibodies in human EBV-carriers or even artificial antibodies when animals are immunized with full length LMP1. However, on the basis of classical structural models of LMP1 and LMP2, one can predict that these external loops are joined side by side and condensed in a very small space. We postulated that this spatial distribution could allow the formation of single epitopes encompassing the amino-acids of two adjacent loops. These putative epitopes were called "inter-loop" epitopes In order to test this hypothesis, we designed oligopeptides containing amino-acids of loop 1 and loop 2 (L1-L2) or loop 2 and loop 3 (L2-L3) of LMP1. These oligopeptides were coupled to KLH and used for immunization of mice and rabbits. They have been found to be highly immunogenic in both species. The resulting polyclonal sera were shown by flow cytometry to react with some live EBV-infected cells known to express LMP1. If the ability of these antibodies to react with native LMP1 is confirmed, the concept of "inter-loop" epitopes will provide the basis for novel passive and/or active immunisation procedures against EBV-associated malignancies.

NOTES:

Poster sessions

Session 11: Drug Development and Therapeutics

216 (RegID: 1166)
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LATENT EBV PROTECTS BL CELLS FROM APOPTOSIS BY AT LEAST TWO DISTINCT PATHWAYS

Jade Yee, Goran Gregorovic, Paul J Farrell, Robert E White and Martin J Allday, Imperial College London

Posterabstract:

Most EBV-negative Burkitt's lymphoma (BL) cell lines rapidly undergo apoptosis in response to various cytotoxic drugs including the cyclin-dependent-kinase inhibitor roscovitine, the DNA cross-linking agent cisplatin and the mitotic spindle poison nocodazole. Previous reports have shown that the latent infection of these BL cells with EBV significantly increased their resistance to apoptosis. This protection is dependent on the co-expression of EBNA3A and EBNA3C, which downregulate the pro-apoptotic protein Bim, but is independent of the tumour suppressor p53 (Anderton et. al., 2008). However, using the calcium ionophore ionomycin as the apoptosis-inducing agent, another pathway by which BL cells undergo apoptosis has been identified. This is independent of both Bim and p53, but can also be blocked by latent EBV.

In order to identify the EBV latent gene(s) responsible for the protection against ionomycin-induced apoptosis, the response of a panel of BL cells expressing restricted sets of viral latent genes was assessed. It was determined that EBNA1, EBNA2, the LMPs and full-length EBNA-LP are not required for this phenotype. Protection was also independent of EBNA3A, EBNA3B or EBNA3C. Experiments conducted using BL cells recently established using new recombinant knockout and revertant EBV-BACs, showed that EBER1, EBER2 and the complete EBNA3 locus are not required for ionomycin-resistance. We also confirmed that this EBV-mediated protection of BL cells did not involve the induction of the viral lytic cycle. Recombinant EBVs are being constructed to determine the role of BHRF1 and the BHRF1-miRNA cluster in the cellular response to ionomycin. Real-time quantitative RT-PCR and Western blot analyses indicate that the pro-apoptotic protein Noxa is consistently induced in BL cells sensitive to ionomycin. The data suggest that EBV confers resistance against ionomycin-induced apoptosis by inhibiting the accumulation of Noxa, via a yet to be identified mechanism.

217 (RegID: 1181)
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SUBEROYLANILIDE HYDROXAMIC ACID (SAHA) INDUCES VIRAL LYTIC CYCLE IN EPSTEIN-BARR VIRUS (EBV) - INFECTED GASTRIC CARCINOMA CELLS AND MEDIATES ENHANCED CELL DEATH

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Department of Paediatrics & Adolescent Medicine, Li Ka Shing Faculty of Medicine,
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Posterabstract:
In Epstein-Barr virus (EBV)-associated malignancies, the virus persists in tightly latent forms expressing a restricted number of viral latent proteins. We seek to activate the viral lytic cycle from its latent form in EBV-infected cancer cells by histone deacetylase inhibitors (HDACis), which may lead to development of novel anti-cancer therapeutic strategies.
A panel of HDACis was tested for their ability to activate the viral lytic cycle in EBV-harboring Burkitt, epithelial cancer and lymphoblastoid cell lines of latency I, II and III, respectively. The treated cells were examined for cell proliferation by MTT assay and viral lytic cycle activation by GFP reporter assay, qPCR and western blot analysis. Cell cycle status of the tumour cells was investigated by flow cytometry.
Our results showed that trichostatin A (TSA), sodium butyrate (NaB), valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA) can all significantly induce the lytic cycle of EBV in EBV-positive gastric carcinoma cells (AGS-BX1, latency II) but not in Burkitt lymphoma cells (AK2003, latency I) or lymphoblastoid cells (LCL-1 and -2, latency III). Interestingly, treating the cells with 2.5uM SAHA resulted in a four-fold increase of EBV load. Expression of two immediate early EBV lytic proteins (BZLF1 and BRLF1) and one early lytic protein (BMRF1) was strongly up-regulated. Increased cell death of EBV-positive AGS-BX1 cells compared with that of EBV-negative AGS cells was observed at this concentration. The enhanced killing of EBV-infected tumour cells may be due to cell cycle arrest at G2 phase.
In conclusion, differential effects of HDACis in the induction of viral lytic cycle in the three forms of EBV latency are found, with the strongest effect seen in latency II in an epithelial cell background. SAHA is found to be a potent viral lytic cycle inducing agent in EBV-positive gastric carcinoma cells and mediates enhanced cell death.

218 (RegID: 1225)
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LMP1 REGULATES THE LIPID PHOSPHATASE, SGGP1 AND THE LEVELS OF SPHINGOSINE-1-PHOSPHATE, A POTENTIAL THERAPEUTIC TARGET IN HODGKIN'S LYMPHOMA.

Baumforth KRN, Reynolds GM, Young LS, Rowe M, Woodman CBJ, Murray PG

Posterabstract:

Sphingosine-1-phosphate (S1P) is an extracellular signaling lipid that promotes cellular proliferation and migration; it can also activate survival and angiogenic pathways. The balance between the levels of pro-apoptotic lipids, such as ceramide, and S1P provides a rheostat mechanism that determines whether a cell is sent into the death pathway (via ceramide) or is protected from apoptosis by S1P. Cancer cells exploit the sphingolipid rheostat by promoting conditions that favor the production of S1P, either through the upregulation of enzymes that promote S1P generation, or by the downregulation of those that degrade S1P, such as SGPP1.

In this study, we have shown that when compared to GC B cells, the presumed progenitor of HL, SGGP1 expression is decreased in both HL cell lines and in primary HRS cells. EBV infection down-regulates SGGP1 in a variety of B cell backgrounds including primary germinal centre B cells, HL cell lines, and Burkitt's lymphoma cell lines; this down-regulation can in part be recapitulated by the ectopic expression of LMP1 in these cells. EBV-induced down-regulation of SGPP1 in HL cells is followed by increased levels of S1P. Increased levels of S1P in turn increase the proliferation and extend the survival of HL cells. EBV-induced increases in S1P could contribute to the immortalisation of B cells and to the pathogenesis of virus-associated cancers, such as HL. We are currently investigating the potential therapeutic targeting of S1P in these cancers using ASONEPTM, a humanised S1P-specific monoclonal antibody.

219 (RegID: 1227)
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THE EPSTEIN-BARR VIRUS PROTEASE : SEQUENCE VARIABILITY, mRNA EXPRESSION AND INHIBITION BY SMALL INTERFERING RNA

Sylvie LARRAT, Patrice MORAND, Ariane BAS, Solenne VIGNE, Jean-Marc CRANCE, Monique BOYER, Sandrine NICOD, Laurence GROSSI, Marlyse Buisson, Wim P BURMEISTER, Jean-Marie SEIGNEURIN and Raphele GERMI

Posterabstract:
EBV lytic replication is necessary for life long viral persistence and inter individual- transmission and may be involved in EBV-associated diseases. EBV protease (EBVPR) is an essential protein of the replication cycle allowing maturation of the viral capsid and DNA packaging. Like the other herpesvirus serine proteases, EBVPR could represent a new target for the inhibition of EBV replication. To date, no data has been reported on the variability and expression in vivo of EBVPR and on the inhibition of EBVPR mRNA by small interfering RNA (siRNA). In this study, the entire EBVPR genes have been sequenced in 39 samples obtained from cell lines or clinical samples. 16/39 harbored 2 constantly associated, amino-acid mutations (aspartic acid changed by a valine in position 77 and valine changed by a leucine in position 105). These mutations were linked neither with the clinical status of the EBV carriers nor with EBV subtypes. Overall, the EBVPR gene is conserved with a 99% amino-acid homology between the different EBV strains. Real time RT-PCR detected the EBVPR mRNA in 3/6 of the AIDS-related B non Hodgkin lymphoma biopsies and 2/7 of the undifferentiated nasopharyngeal carcinoma biopsies. EBVPR mRNA detection was associated with ZEBRA and gp350/220 lytic mRNAs. siRNA targeting EBVPR in the epithelial 293 cells stably transfected with the complete B95-8 EBV episome demonstrated : (i) a 73% reduction of the EBVPR mRNA amount compared to a irrelevant control siRNA; (ii) a drastic decrease (>95%) of the infectious virus titers as measured by super-infection of Raji cells. This inhibitory effect was dose-dependant and inhibitory concentration 50% was 0.55nM for mRNA inhibition and 0.09nM for virus titer inhibition. In conclusion, the siRNA strategy targeting EBVPR mRNA to inhibit EBV replication could represent a new strategy against EBV-associated diseases.

220 (RegID: 1290)

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TWO STEP EPSTEIN - BARR VIRUS IGA-ELISA SYSTEM FOR SEROLOGICAL SCREENING AND CONFIRMATION OF NASOPHARYNGEAL CARCINOMA.

Dewi K. Paramita1, Jajah Fachiroh1,Sofia M. Haryana1 and Jaap M. Middeldorp2.

Posterabstract:

Undifferentiated nasopharyngeal carcinoma (NPC, WHO type-III) is 100% associated with Epstein-Barr virus (EBV) infection and the 4th most prevalent cancer in Indonesian males. Therapy failure is high, since most patients come to the hospital at advanced stage of disease. Screening for early-stage NPC is needed. Here, a simple, standardised and economical 2-step ELISA system is proposed for diagnosing NPC in high risk populations, employing peptide-based IgA-[EBNA1+VCAp18] ELISA (Fachiroh et al., 2006) as initial screening test and IgA-EA ELISA (Paramita et al., 2007) using a different set of EBV antigens as confirmation test.

151 NPC patients and 199 regional healthy EBV carriers were used to evaluate the 2-step ELISA approach. Routinely EBV IgG immunoblot is used as standard confirmation test (Fachiroh et al., 2004). The sensitivity and specificity for diagnosing NPC by the 2-step ELISA approach increased from 85.4% to 96.7% and 90.1% to 98% respectively, with PPV/NPV values increasing from 78.7/93.9% to 97.3/97.5%, relative to the immunoblot confirmation system. On discrepant samples, additional testing was done by EBV DNA load quantification in blood (Stevens et al., 2005). Result showed that 5/11 discrepant NPC samples with elevated IgA-EA ELISA also had elevated EBV DNA load in the circulation (range 3,200-25,820 copies/ml). Therefore IgA-EA ELISA is proposed as confirmation test in first-line NPC serological screening studies. This 2-step EBV-ELISA system provides a standardized approach for NPC screening, and may be used in combination with dried-blood sampling in future field studies for identification of early stage NPC in high-risk regions (Fachiroh et al., (2008).

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221 (RegID: 1466; 1467)

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HETEROGENEOUS RIBONUCLEOPROTEIN K AND THYMIDINE PHOSPHORYLASE ARE INDEPENDENT PROGNOSTIC AND THERAPEUTIC MARKERS FOR NASOPHARYNGEAL CARCINOMA

Lih-Chyang Chen, Chuen Hsueh, Ngan-Ming Tsang, Ying Liang, Kai-Ping Chang, Sheng-Po Hao, Jau-Song Yu, Yu-Sun Chang

Posterabstract:

Purpose: Heterogeneous ribonucleoprotein K (hnRNP K) regulates thymidine phosphorylase (TP) mRNA stability. The aim of the present study was to analyze hnRNP K and TP expression in nasopharyngeal carcinoma (NPC) and to evaluate the prognostic and therapeutic potential of these two markers.

Experimental Design: We analyzed hnRNP K and TP expression immunohistochemically in 121 clinically proven NPC cases. Statistical analyses were applied to correlate cytoplasmic hnRNP K with elevated TP expression and determine the prognostic significance of these parameters. The therapeutic implication of elevated TP expression was determined by measuring sensitivity of NPC cells to the TP-targeting drug, 5-Fluoro-5’-deoxyuridine (5'dFUrd).

Results: There was a high correlation between cytoplasmic hnRNP K and high TP ($P < 0.001$). Both cytoplasmic hnRNP K and high TP were associated with poor overall survival (OS) ($P = 0.007$ and $P < 0.001$, respectively) and distant metastasis-free survival (DMFS) ($P = 0.003$ and $P = 0.001$, respectively) of NPC patients. A multivariate analysis confirmed that both cytoplasmic hnRNP K and high TP are independent prognostic predictors for OS ($P = 0.020$ and $P = 0.010$, respectively). NPC cells expressing high TP were more sensitive to treatment with the TP-targeting drug, 5'dFUrd.

Conclusions: Cytoplasmic hnRNP K and high TP are associated with shorter OS and DMFS in NPC patients. In vitro experiments suggest that NPC tumors with high TP expression may be sensitive to 5'dFUrd treatment. Cytoplasmic hnRNP K and high TP may be potential prognostic and therapeutic markers for NPC, but additional validation studies are warranted.

222 (RegID: 1591)
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STUDY ON EPSTEIN-BARR VIRUS AND HUMAN HERPES VIRUS 6 INFECTION IN DRUP ERUPTION PATIENTS

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Posterabstract:
Drug eruption，a drug-induced systemic reaction to certain drugs，is a kind of common emergency case in departmrnt of dermatology. It has serious symtoms，associated with the following manifestations：high fever over 38℃，lymphadenopathy，facial edema，marked leukocytosis with eosinophilia and atypical lymphocytes，lymphocytopenia, liver and renal dysfunction，and low IgG level．However, the machanism of drup eruption is not wellknown．Our experiment was to study the role of Epstein-Barr virus and Human herpes virus 6 in drug eruption patients．EBV genome were tested in PBMC of 31 drup eruption patients and normal control by PCR-Southern blotting．Our result suggested that the positive rate of EBV DNA in drup eruption patients was higher than in normal control（$P<0.01$）．HHV6 DNA was tested by nested-polymerase chain reation，10 of 31 drug eruption patients were HHV6 positive，then the positive samples were genotyped，it was interesting that all of HHV6 positive samples were HHV6A，we could presume that HHV6A plays an important role in the the progress of drug eruption，there was no obvious difference about the positive rate of HHV6 between the two groups（$P>0.05$）．ELISA result showed that the positive rate of EBV VCA-IgM in drup eruption patients was higher than the normal control（$P<0.05$）．3 EBV BZLF1 positive samples were tested by RT-PCR-Southern blotting in EBV positive samples，2 of 3 EBV BZLF1 positive samples were HHV6 positive．9 of 10 HHV6 positive samples were EBV positive．From the data above we could suppose that EBV and HHV6 could activate each other，latent virus are reactivated in drug eruption patients．

223 (RegID: 1744)

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GANCICLOVIR MEDIATED INHIBITION OF EBV REPLICATION REQUIRES THE VIRALLY ENCODED KINASE (EBV-PK)

Qiao Meng, Stacy Hagemeier, Shannon C. Kenney

Posterabstract:

Ganciclovir (GCV) is a synthetic analogue of 2'-deoxy-guanosine that effectively inhibits the lytic form of viral replication of a number of different herpesviruses. GCV has also been used as a lytic protein-activated prodrug to kill EBV-positive tumor cells when combined with lytic inducing agents. Ganciclovir cannot be incorporated into viral or cellular DNA unless it is phosphorylated and converted into a nucleotide. In CMV-infected cells, the viral kinase (UL97) mediates GCV phosphorylation, whereas in HSV- infected cells, GCV phoshorylation is mediated by the viral thymidine kinase. Whether GCV phosphorylation in EBV-infected cells is primarily mediated by the BGLF4 gene product (EBV-protein kinase), the EBV-encoded thymidine kinase (TK), or requires both remains unknown. In this report, we examined the ability of GCV to inhibit viral replication in wild type virus infected 293T cells, versus 293T cells infected with EBV mutants containing inserted stop codons in either the EBV-PK or EBV-TK open reading frames. Lytic replication was induced by transfecting cells with a BZLF1 expression vector, and viral titer was quantitated in the supernatant using the green Raji cell assay. As expected, GCV (5 µg/ml) efficiently inhibited virus production in wild type virus-infected 293T cells. However, an EBV mutant unable to express the viral PK (which can replicate in 293T cells) was highly resistant to GCV. Expression of the PK protein in trans restored GCV sensitivity in cells infected with the PK-mutant virus. In contrast, GCV efficiently inhibited viral replication in 293T cells infected with the TK-mutant virus. The PK-mutant virus also had reduced susceptibility to acyclovir (although the effect was less dramatic than the GCV effect). These results suggest that GCV phosphorylation in EBV-infected cells requires the EBV-encoded protein kinase, and that PK may also contribute to acyclovir phosphorylation.

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THE SRC FAMILY KINASE, LYN, NEGATIVELY REGULATES AKT ACTIVATION IN LMP2A-EXPRESSING CELLS

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Oralabstract:

The EBV protein, LMP2A, is critical for maintaining viral latency and provides pro-survival and migration signals for EBV-positive B and epithelial cell malignancies. LMP2A's ability to initiate signaling was initially proposed to proceed via a two-step mechanism. Firstly, recruitment of the Lyn tyrosine kinase to the tyrosine phosphorylated YEEA site in LMP2A allows for tyrosine phosphorylation of the LMP2A ITAM. This, in turn, facilitates the recruitment and activation of the Syk tyrosine kinase, which initiates downstream signaling events. However, recent findings suggest that Syk could be recruited to LMP2A independent of the YEEA site. Therefore, we undertook experiments to clarify the role of the YEEA motif and Lyn in initiating LMP2A signaling in B lymphocytes. We found that the YEEA site was not required for LMP2A ITAM tyrosine phosphorylation, or for LMP2A to activate Syk. Using siRNA to silence Lyn expression or Lyn-deficient chicken DT40 B cells, it was observed that ITAM and Syk tyrosine phosphorylation did not require Lyn in LMP2A-expressing cells. Furthermore, Lyn was not required for LMP2A-mediated Akt activation in DG75 B cells, but rather Akt activation was significantly enhanced in LMP2A-expressing cells in which Lyn was reduced. We propose that Lyn negatively regulates LMP2A-mediated Akt activation by phosphorylating tyrosine 323 of Syk, which serves to recruit the c-Cbl E3 ubiquitin ligase to Syk and targets Syk for ubiquitin-mediated degradation. In sum, this work demonstrates that LMP2A can initiate signaling independent of Lyn and that Lyn can negatively regulate LMP2A signaling.

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Final Program and Abstracts