Karolinska Institutet

Department of Microbiology, Tumor and cell biology

THE IMPACT OF MICROENVIRONMENTAL FACTORS ON EBV CARRYING B CELLS

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Nobels väg 12B, Atrium

Måndag den 29 April, 2013, kl 14.00

av
Liang Wu

Huvudhandledare:
Professor Ingemar Ernberg
Karolinska Institutet
Department of Microbiology,
Tumor and cell biology

Bihandledare:
Professor George Klein
Karolinska Institutet
Department of Microbiology,
Tumor and cell biology

Docent Elena Kashuba
Karolinska Institutet
Department of Microbiology,
Tumor and cell biology

Fakultetsopponent:
Professor Riccardo Dolcetti
National Cancer Institute, Italy

Betygsnämnd:
Docent Theresa Frisan
Karolinska Institutet
Department of Cell and Molecular Biology

Professor Matin Rottenberg
Karolinska Institutet
Department of Microbiology,
Tumor and Cell Biology

Professor Christer Sundström
Uppsala University
Institutionen för Genetik,Patologi,
Immunologi

Stockholm 2013

ABSTRACT

Epstein-Barr virus (EBV) is a human gamma-herpes virus that colonized more than 90% of the adult population. The virus is able to infect and immortalize B lymphocytes both *in vitro* and *in vivo*. Despite of the mostly harmless outcome of the EBV infection, EBV is associated with a number of malignancies, such as Burkitt lymphoma, classical Hodgkin lymphoma, and Diffuse large B-cell lymphoma (DLBCL).

DLBCLs, the most common group of malignant lymphomas, account for 30% of adult non-Hodgkin lymphomas (NHLs). EBV-positive DLBCL of the elderly is a newly recognized subtype of DLBCL which accounts for 8% to 10% of DLBCL in Asian countries, but seems to be less common in Western populations.

In this study we have characterized EBV-positive DLBCL cell lines by checking EBV latent gene and cellular gene expression. Then, we studied the modulation of EBV latent gene and cellular gene and the EBV modulated chemotaxis in the EBV-positive DLBCL lines upon cytokine treatment. We found that IL-4 and IL-21 upregulated LMP1 expression in EBV-positive DLBCL lines and IL-21 downregulated EBNA2 and EBNA1 expression in the type III line, Farage. IL-4 and IL-21 were found to induce different patterns of CXCR4 or CCR7 mediated chemotaxis in DLBCL lines. We also knocked out EBV from EBV-positive DLBCL lines and found that EBV provided survival factors to these lines. We further studied modulation of chemotaxis after downregulation of EBV encoded genes by dominant negative EBNA1 (dnEBNA1) in DLBCL cells upon cytokine treatment and observed decreased chemotaxis mediated by CXCR4 or CCR7 upon IL-4 or IL-21 treatment.

As IL-21was reported to induce apoptosis in DLBCL lines with unknown EBV status, we also examined the IL-21 sensitivity of the EBV positive type III DLBCL line, Farage, and found surprisingly that despite c-Myc upregulation, IL-21 induced cell proliferation rather than apoptosis. EBV knock-out counteracted the IL-21 induced proliferation of Farage and increased apoptosis. This finding reveals a previously unknown role of EBV in DLBCL that is of possible relevance for the current attempt to use IL-21 in therapy.

Studies on the EBV modulated chemotaxis after EBV infection on tonsillar B cells found downregulation of CXCR5 and CCR7 mediated chemotactic responses, which are important for migration into lymphoid tissue. These alterations may lead to retention of EBV-infected tonsillar B cells in the interfollicular region of the tonsil.

Further work on type I interferons (IFNs) identified their role in upregulation of LMP1 expression by direct activation of the ED-L1 promoter in several EBV-carrying Burkitt's lymphoma lines. In EBV-infected primary B cells, IFN-α transiently upregulated LMP1 mRNA, but not protein levels, followed by downregulation of both, suggesting a novel antiproliferative mechanism of type I IFNs.

Altogether our results not only provide evidence for the important roles of microenvironmental stimulation in EBV-carrying B cells but might also have future therapeutic implications.

ISBN 978-91-7549-134-9