Dissertation Abstract

Regulation of the Epstein-Barr Virus Latent Membrane Protein 1 Expression

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Epstein-Barr virus (EBV) is a probably the most effective and successful human virus, infecting more than 90% of the world's adult population. As with the other members of the herpesvirus family, EBV establishes latent infection in its host and persists life-long. EBV infection is generally harmless in children but can cause infectious mononucleosis (IM) in young adults. EBV is associated with a number of human malignancies including Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), nasopharyngeal carcinoma (NPC), nasal T/NK lymphoma (NL), peripheral T cell lymphoma, gastric carcinoma, and lymphoproliferative diseases in immunocompromised patients. A compromised immune system and an aberrant EBV latent gene expression are thought to be important players in the aetiology of EBV malignancies. EBV is one of the most potent transforming agents in vitro and immortalizes B cells into lymphoblastoid cell lines (LCLs).

Latent membrane protein 1 (LMP1) is the main EBV oncogene, which is critically involved in immortalisation and proliferation of LCLs, and is associated with most EBV malignancies. LMP1 functions as a constitutively active tumour necrosis factor receptor (TNFR) and upregulates anti-apoptotic and pro-survival proteins through the activation of cellular signalling pathways. Thus, inappropriate expression of LMP1 is probably a central process in EBV associated tumourigenesis. The aim of this PhD project was to delineate the regulation of LMP1 gene expression in response to cellular factors.

The LMP1 protein expression is regulated differently according to the expression pattern of the other EBV latent proteins as well as the cell type in which it is expressed in. In latency III infected B cells all of the EBV latent proteins are expressed, and LMP1 expression is driven by the viral transcription factor EBNA2. The EBNA2 protein lacks DNA binding ability itself, and requires cellular factors (adaptors) to be recruited to promoters. In latency II cells that represent most EBV tumours and different cell-type hosts, a more limited set of EBV latent proteins are expressed, and LMP1 expression occurs in the absence of EBNA2. Regardless of the mode of expression and cell type, LMP1 transactivation is critically dependent on cellular proteins.

In the course of this investigation, a new EBNA2 adaptor was identified that bound an AP-2 site in the LMP1 promoter and mediated the relief of promoter repression and activation of the LMP1 promoter.

We also report EBNA2-independent upregulation of the LMP1 promoter in response to upregulation of the p38 kinase pathway. The p38 signalling pathway activates the ATF1-CREB heterodimer that has been previously shown as an activator of LMP1 transcription. The binding of ATF1-CREB to a CRE site is a central event in LMP1 regulation both in the presence and absence of EBNA2.

Additionally, we showed the presence of a mutation in the LMP1-CRE site of the P3HR1 EBV variant. This mutation led to a reduced binding efficiency of ATF1-CREB to the CRE site and a two fold reduction of LMP1 promoter activity. This finding together with reports from other groups indicate that sequence variations in the CRE site of LMP1 are evolutionary, selected probably to modulate the expression levels of the protein.

Our results also indicate that the NF-κB dimers, p50-p65 and p50-p50, bind an NF-κB site in the LMP1 promoter and activate transcription independently of EBNA2. The EBNA2-independent activation of LMP1 transcription by NF-κB suggests that this signalling pathway may play a role in LMP1 activation in latency II infected B cells. Since the NF-κB pathway is activated by LMP1, a positive autoregulatory loop in LMP1 activation may exist. The positive autoregulation of LMP1 is supported by reports from other groups.

Finally, we showed that histone acetylation and modulation of the chromatin structure of the LMP1 promoter are involved in the activation of LMP1 transcription. We hypothesise a model whereby the EBNA2 is recruited through interaction with several EBNA2 adaptors at the promoter and mediates activation. Alternatively, several transcriptional activators such as NF-κB factors and ATF1-CREB bind the promoter in the absence EBNA2 and cooperatively activate the promoter. In both cases factor-binding to the promoter leads to the recruitment of histone acetylases and chromatin remodelling enzymes to the LMP1 promoter to facilitate transcription.

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II. Jansson A, Johansson P, Yang W, Palmqvist L, Sjöblom-Hallén A and Rymo L. (2007). **Role of a consensus AP-2 regulatory sequence within the Epstein-Barr Virus LMP1 promoter in EBNA2 mediated transactivation**. *Virus Genes*, 35, 203-14.

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