Characterization of non-coding mRNA in Epstein-Barr virus

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av

Åsa Isaksson

Avhandlingen baseras på följande arbeten:

I **Epstein-Barr virus U leader exon contains an internal ribosome entry site**
Åsa Isaksson, Malin Berggren and Anne Ricksten.
Oncogene (2003) 22, 572-581

II **Alternative EBNA1 expression in organ transplant patients**
MalinÅ.M. Berggren, Åsa Isaksson, Ulrica Larsson, Folke Nilsson, Ulla Nyström, Tor Ekman, Jane Löfvenmark and Anne Ricksten.
Journal of Medical Virology 76:378-385 (2005)

III **Cell specific internal translation efficiency of Epstein-Barr virus present in solid organ transplant patients**
Åsa Isaksson, Malin Berggren, Kerstin-Ekeland-Sjöberg, Tore Samuelsson and Anne Ricksten.
Journal of Medical Virology 79:573-581 (2007)

IV **Interactions of cellular proteins with the EBV internal ribosome entry site**
Åsa Isaksson, Malin Berggren and Anne Ricksten.
In manuscript

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Dissertation abstract

Characterization of non-coding mRNA in Epstein-Barr virus

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Epstein-Barr virus (EBV) is a human gammaherpesvirus that infects lymphoid and epithelial cells. The virus is the causative agent of infectious mononucleosis, a self-limiting lymphoproliferative disease, and it is additionally associated with various malignancies including Burkitt's lymphoma, Hodgkin's disease and lymphoproliferative syndromes in immunocompromised individuals. The Epstein-Barr virus nuclear antigen 1 (EBNA1) is the only EBV protein expressed in all known states of EBV latency and in the virus lytic cycle. EBNA1 is required for the replication and maintenance of the EBV episome. The aim of this thesis was to characterize non-coding mRNA in EBV, with the focus on EBNA1 gene regulation.

We identified an internal ribosome entry site (IRES) in the 5' untranslated region (5' UTR) of the EBNA1 mRNA. This element, designated EBNA IRES, promotes cap-independent translation by recruiting ribosomes directly to highly structured internal mRNA regions and was shown to increase EBNA1 protein expression.

EBNA1 expression and regulation in peripheral blood cells from organ transplant patients were characterized by RT-PCR and Southern blotting. These patients are at high risk for developing EBV-associated post transplant lymphoproliferative disease (PTLD). The incidence of EBNA1 expression in samples from PTLD patients was 3-fold higher compared to other transplant recipients. In addition to the normal EBNA1 transcript we found an alternatively spliced transcript in the transplant recipients. This transcript was shown to exclude the EBNA IRES element and will consequently not promote IRES mediated translation.

Nucleotide changes were found in the patient derived EBNA IRES mRNA compared to the EBNA IRES derived from the laboratory EBV strains B95.8 and Rael in one or two positions, respectively. The patient specific sequence significantly decreased the IRES activity in T cells, while the nucleotide changes had no significant impact on the activity in B or in epithelial cells.

The ability of EBNA IRES to bind cytoplasmic proteins was examined with electrophoretic mobility shift assay (EMSA). Protein-RNA complexes were identified, showing that the EBNA IRES interact specificially with cytoplasmic proteins collected from both EBV-positive and -negative cell lines. With EMSA competition experiments we showed that the patient specific EBNA IRES bound more efficient to trans-acting proteins compared to the B95.8-derived EBNA IRES.

In summary, we have provided evidence that IRES activity, alternative splicing of non-coding mRNA and nucleotide changes in the EBV genome are important mechanisms for translational control of EBV latent gene expression.

Keywords: *Epstein-Barr virus, EBNA1, IRES, PTLD, alternative splice, nucleotide substitution, protein interactions*

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