Regulation and expression of Epstein-Barr virus nuclear antigen 1 in transplant patients and cell culture

AKADEMISK AVHANDLING
Som för avläggande av medicine doktorsexamen vid Göteborgs universitet kommer att offentligen försvaras i Sahlgrenska sjukhusets aula,
torsdagen den 17:e april 2008, kl 9:00

av

Malin Berggren

Avhandlingen baseras på följande arbeten:

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

II. Alternative EBNA1 expression in organ transplant patients
Berggren M, Isaksson Å, Larsson U, Nilsson F, Nyström U, Ekman T, Löfvenmark J, Ricksten A. *J Med Virol (2005) 76(3): 378-385.*

III. EBNA1 expression in a lung transplant recipient with hypocomplementemic urticarial vasculitis syndrome
Berggren M, Heinlen L, Isaksson Å, Nyström U, and Ricksten A. *J Med Virol (2007) 79(7): 963-969.*

IV. EBNA IRES mediates translation during lytic induction of Epstein-Barr virus
Berggren M, Jasinska A, Isaksson Å and Ricksten A. *Manuscript 2008*

Fakultetsopponent: Professor Ingemar Ernberg, Institutionen för mikrobiologi, tumör- och cellbiologi, Karolinska Institutet, Stockholm, Sverige

Dissertation Abstract

Regulation and expression of Epstein-Barr virus nuclear antigen 1 in transplant patients and cell culture

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Epstein-Barr virus (EBV) is a human herpes virus that infects over 90% of the world population. Once infection has occurred, the virus persists for life in its host, mainly in an asymptomatic, latent stage with only a few active viral genes. In immunosuppressed transplant patients, the virus is sometimes reactivated and may cause cell proliferation with the risk of developing post transplant lymphoproliferative disorder (PTLD).

Epstein-Barr virus nuclear antigen 1 (EBNA1) is important for virus replication and segregation in dividing cells and it is the only viral protein expressed in all dividing B cells and is therefore a key to monitoring the virus and possibly to detect early changes in viral activity.

This thesis focuses on regulation of EBNA1 expression in transplant patients and the discovery of an internal ribosome entry site (IRES) in the 5´ untranslated region of EBNA1 gene. The EBNA IRES enables translation of a downstream gene even if regular cap-dependent translation is impaired. We establish that the EBNA IRES main activity is located within the U exon of the EBV genome. This exon with its IRES function seems to be very important for EBV since it is also part of the EBNA 3, 4 and 6 transcripts (Paper I).

Expression of EBNA1 in peripheral blood is undetectable in a healthy population but may be demonstrable in immunosuppressed individuals. A comparison between transplant patients who were diagnosed with PTLD and transplant patients without these symptoms, showed a more than three fold incidence of EBNA1-expression in blood from PTLD patients. Detection of EBNA1 in peripheral blood may therefore be used in risk evaluation for post transplant lymphoproliferative disease among transplant recipients. We also found that the EBNA IRES is sometimes deleted in the process of mRNA alternative splicing in transplant patients, as we discovered from EBNA1 expression analysis. These transplant patients express both regular and alternatively spliced EBNA1 mRNA. This finding implicates a new model for EBV translational regulation through the deletion of an IRES element (Paper II).

Further we recognized a lung transplant recipient, with no sign of PTLD, who persistently expressed EBNA1 in peripheral blood. This patient has a rare underlying autoimmune disease called hypocomplementemic urticarial vasculitis syndrome (HUVS). HUVS is closely related to systemic lupus erythematosus, which has previously been proposed to be associated with EBV via autoimmune, cross-reactive antibodies against EBNA1. In this case study, we explored the possibilities for a similar connection between HUVS and EBV and found increased antibody response to EBNA1 epitopes in the patient serum when compared to sera from a matched transplant control and healthy blood donors. This is the first study of EBV expression in the HUVS context and further studies are needed to investigate the role of EBV in this disease (Paper III).

In paper IV, the activity of EBNA IRES during lytic induction was investigated. Two reporter vectors were designed, with and without the EBNA IRES, and were stably transfected into EBV-positive B cell lines representing three different types of latency. The transfected cells were induced to enter EBV lytic phase and the effects of the EBNA IRES on the reporter gene expression was studied at the protein and RNA levels. The results showed a 2-3 fold protein expression in induced cells transfected with the EBNA IRES compared with the induced cells with the vector lacking EBNA IRES. These data point to the potential of EBNA IRES activity during lytic EBV infection.

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