The Epstein-Barr Virus Nuclear Antigens 1 & 5
Study of virus-host cellular protein interactions

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i hörsal Hjärtat, Sahlgrenska universitetssjukhuset, fredagen den 4:e december 2009, kl. 9:00

av

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Avhandlingen baseras på följande arbeten:

I. **Identification of intracellular proteins associated with the EBV-encoded nuclear antigen 5 using an efficient TAP procedure and FT-ICR mass spectrometry.**
Forsman, A., Rüetschi, U., Ekholm, J. and Rymo, L.
Journal of Proteome Research (2008) 7: p. 2309-19

II. **Epstein-Barr virus nuclear antigen 5 is a multi-functional protein with a possible role in the chaperone-mediated protein folding and ubiquitin-proteasome protein degradation systems.**
Ekholm, J., **Forsman, A.,** Kashuba, E., Andersson, M., Rüetschi, U. and Rymo, L.
In manuscript (2009)

III. **E2F1, ARID3A/Bright and Oct-2 factors bind to the Epstein-Barr virus C promoter, as well as to EBNA1 and *oriPI*, possibly facilitating long-distance promoter-enhancer interactions.**
Boreström, C., **Forsman, A.,** Rüetschi, U., and Rymo, L.
In manuscript (2009)

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The Epstein-Barr virus (EBV) is the causative agent or cofactor in the aetiology of several human malignancies such as Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma (NPC) and lymphoproliferative disorder in immunocompromised patients. EBV is a lymphotropic γ-herpes virus infecting more than 90 percent of the population worldwide. Following acute infection the virus establishes a life-long latency in resting memory B cells. The virus is remarkable for the efficiency with which it causes proliferation and immortalization of the infected B cells through expression of several latent gene products. All of the viral EBNA proteins have been proposed to play a role in the control of gene expression in the EBV infected lymphoblastoid cell.

The present thesis is mainly focused on further elucidating the molecular mechanisms of the EBNA1 and EBNA5 proteins using proteomic technologies as approach. In paper I we used an improved tandem affinity purification procedure for identification and characterization of factors in the EBNA5 interaction proteome. The majority of the 37 validated interactors could be assigned to one of three groups according to function: protein folding and degradation, pre-mRNA processing, or ribosomal proteins, implicating functional relationships with EBNA5 in these processes. We also showed that EBNA5 is part of high molecular protein complexes, supporting the notion that functional units in the cell are not single proteins but well-structured complexes composed of multiple proteins i.e. modules.

The previously reported repressor activity of EBNA5 was further investigated in paper II. The study identified the novel interactor BAG2 as a major target for the function of EBNA5 via the chaperone-mediated folding and proteasome-degradation pathways. Taken together, the results are consistent with the hypothesis that EBNA5 tune the balance between protein rescue and destruction in a way that disfavour the path of degradation.

The constituents of the large macromolecular complex that initiates transcription from the viral C promoter were investigated in paper III. Using a DNA affinity procedure we showed that the transcription factors E2F1, ARID3A/Bright and Oct-2 binds the Cp as well as EBNA1 and *oriPI*, possibly facilitating long-distance promoter-enhancer interactions.

While the study of genes and proteins continues to be important, looking at isolated components is not enough to understand most biological processes. Modularity has been proposed as a general principle for the molecular architecture of living systems. These assemblies interact with other large protein complexes, thus the proteins are part of a protein-protein interaction network inside the cell. A common feature of these interaction networks is that it contains junctions of proteins that are highly interconnected, also called hubs. Hubs have a tendency of being essential and involved in cancer development. Two central pathways in cancer biology are the Rb- and p53-pathways, which are targets for both EBNA1 and EBNA5 action. This is consistent with the hypothesis that several viral proteins target the same hubs in the host, which ensures the takeover of the cellular machineries essential for the viral infection and persistence processes, and contribute to the robustness of the viral infectious system.

Keywords: *Epstein-Barr virus, EBNA1, EBNA5, EBNA-LP, cellular network, virus-host protein interactions*

ISBN 978-91-628-7948-8