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Modulation of immune response during herpesvirus infection - molecular aspects of BHV-1-encoded UL49.5/gM protein complex activity

Bovine herpesvirus 1 (BHV-1, BoHV-1) is an economically important pathogen of cattle. What is more, due to its close relationship with human herpesviruses, like varicella-zoster virus (VZV) and herpes simplex virus (HSV), BHV-1 is believed to be a good and safe model for herpesvirus infection.

Herpesviruses that remain in an infected organism through its entire life, have evolved several strategies to avoid the host immune response. Antigenic peptide presentation via major histocompatibility complex proteins (MHC) is one of the central targets of herpesvirus immune evasion. The main focus of this work was BHV-1-encoded UL49.5 protein, a key player in immune evasion mechanisms employed by the virus and some of its close relatives. UL49.5 protein down-regulates MHC I expression on the surface of infected cells and, in consequence, impedes their recognition by cytotoxic T lymphocytes (CTLs). UL49.5 targets the transporter associated with antigen processing (TAP), which is responsible for antigenic peptide translocation from the cytosol to the endoplasmic reticulum (ER), where loading on maturing MHC I occurs.

BHV-1-encoded UL49.5 is a small transmembrane type I protein, composed of an N-terminal domain located in the ER, a transmembrane region and a cytosolic domain. In the cell, UL49.5 appears in two major forms: as a monomer and in a complex with another BHV-1-encoded protein: glycoprotein M (gM). Complex formation regulates the activity of both proteins through their inter-dependent folding, ER-release and subcellular transport. The gM-unbound form of UL49.5 is retained in the ER, where it blocks TAP and causes its degradation. Mature complex participates in viral egress and cell-to-cell spread.

The main aim of the project was to explore the molecular mechanism of BHV-1-encoded UL49.5/gM complex activity in two, previously unknown, aspects. Firstly, based on the UL49.5 structural model, the impact of selected N-terminal amino acid residues and structural motifs on UL49.5-mediated TAP inhibition and degradation was analyzed. I tested the effect of charged residues potentially involved in salt bridge formation and proline residues that create a β-turn within the UL49.5 structure. Designed substitutions were introduced into the UL49.5 sequence by site-directed mutagenesis. Next, the activity of UL49.5 variants was examined in human melanoma (MJS) cells transduced with retroviral vectors or in MJS or bovine kidney MDBK cells infected with constructed BHV-1 mutants. I could demonstrate that N-terminal proline residues of UL49.5 are crucial for effective TAP inhibition. What is more, their substitution resulted in alterations of the entire UL49.5 structure. In this aspect the hypothesis relating the UL49.5 activity to its structural motifs was

confirmed. The collected data suggest the important role of salt bridges formation in UL49.5 folding and/or interaction with TAP, since modification of selected residues charge resulted in decreased TAP degradation. I also questioned the role of important ER chaperone BiP in the UL49.5-mediated inhibition of TAP.

In the second part of the project I focused on the identification of crucial motifs and residues in gM and UL49.5 involved in complex formation, ER-release and maturation, as mechanisms controlling UL49.5 activity. The obtained results indicate that N-terminal domain of UL49.5 is sufficient to form a complex with gM, yet its transmembrane region can regulate this interaction. What is more, I found the presence of noncovalent interactions, probably between the transmembrane regions of investigated proteins, strong enough to stabilize the complex. In addition, UL49.5/gM complex formation, its ER-release and maturation was proved to rely on glycine zipper motifs located within transmembrane regions of gM. Detailed knowledge about the UL49.5 residues and structural motifs crucial for TAP inhibition and/or gM binding may contribute to better understanding of the mechanism of UL49.5 activity. It may point directions for virus genetic modifications that could yield more effective vaccine strains or oncolytic vectors for human cancer therapy.