

## **Abstract**

### **Exploitation of pseudocapsids for studies of functions of polyomavirus minor structural proteins VP2 and VP3**

Polyomaviruses are non-enveloped DNA viruses with capsid composite of three proteins – the major VP1 and the minor VP2 and VP3. The function of minor structural proteins remains still unclear in spite of many years of researching. We studied possible function of these proteins in the early phase of infection by using virus-like particles (VLPs) of murine polyomavirus as competitive inhibitors of infectivity. Furthermore, we compared localization and cytotoxic properties of structural minor proteins of BK virus and murine polyomavirus (MPyV) fused with EGFP.

First we infected murine 3T3 fibroblasts by viral inocula mixed with VLPs composite of VP1 proteins only, VP1 and VP2 proteins or VP1 and VP3 proteins. To detect possible inhibition of viral infection by VLPs, we counted cells expressing LT antigen using indirect immunofluorescence and flow cytometry one day post infection. Surprisingly, we did not observe any evidential changes in rate of infection. This result implies there is probably no competition among VLPs and infective virions for extra and intracellular receptors.

Furthermore we compared localization and cytotoxicity of fusion variants of minor proteins VP2 and VP3 (fused C- or N-terminally with EGFP) and minor proteins alone of both viruses expressed in murine 3T3 and simian VERO fibroblasts. All forms of fusion variants have very similar localization like non-fused minor proteins, except for artificial nucleolar localization of BKV with EGFP added to C-terminus. Whereas the cytotoxicity of MPyV proteins fused with EGFP situated on C-terminus resembled non-fused proteins, cytotoxicity of non-fused BKV minor proteins resemble fusion variants with EGFP situated on N-terminus. Taken together, the BKV and MPyV minor proteins have different properties when expressed in cells dependent on protein type (non-fused or N- or C-terminal fused with EGFP), whereas the influence of used cell type was neglectable.

**Klíčová slova:** myší polyomavirus, BK virus, strukturní minoritní proteiny, kompetitivní inhibice, pseudokapsidy (VLPs), apoptóza

**Key words:** murine polyomavirus, BK virus, structural minor protein, competitive inhibition, virus-like particles (VLPs), apoptosis,