Study of functions of VP2 and VP3 minor structural proteins of mouse polyomavirus

ABSTRACT:

The long-term aim of our laboratory is to solve a mechanism of genome delivery by polyomavirus virions. Polyomavirus is a small nonenveloped dsDNA virus composed of major structural protein VP1, minor structural proteins VP2/VP3 and nucleocore. 72 pentamers of VP1 proteins form capside particles. Minor proteins are bound in the inner space of the cavity of each pentamer. Role of minor proteins in polyomavirus life cycle is not clear but they were shown to play a role in very early steps of infection. The aims of this study were: to constructed recombinant plasmids for expression of PyV minor structural protein sequences in *E. coli* and to prepare polyclonal antibody against VP2/VP3 for future investigation: of interactions of minor structural proteins with host cell structures.

Plasmids carrying sequences for entire VP3 and its C terminal 35 amino acids (aa) fused with sequences encoding bacterial thioredoxin and oligohistidin were constructed. Fuse proteins Trx-Histag-VP3, Trx-Histag-C35, Trx-Histag-α helix (Trx-Histag-C17 without C terminus18 aa) and Trx-Histag-VP2 (constructed by E. Bouřa) were produce in bacterial cells and isolated. Protein Trx-Histag-VP2 was used as an antigen for preparation of rabbit polyclonal antibody. This antibody was purified by affinity chromatography and tested by Western blot and by indirect immunofluorescent assays.

Synthetic peptide C35 (prepared at UCHOAB), that contained last 35 aa of the C terminal part of VP2/VP3, was used for investigation of its interactions with the plasma membrane of mammalian cells. It was shown, that peptide C35 is able to interact with the plasma membrane and to entry into cells by both clathrin depended and by caveola dependent endocytosis. Peptide C35 was found to colocalise with early endosomes 15 minutes post adsorption. Internalisation of the peptide was accompanied by cytoskeleton reorganisation.

Key words: Polyomavirus, minor structural proteins, bacterial expression, Protein Transduction Domain (PTD), virus entry,

Klíčová slova: Polyomavirus, minoritní strukturní proteiny , bakteriální expresní systém, PTD, vstup viru do buněk