Studies of delivery of the mouse polyomavirus genome into the cell nucleus

ABSTRACT:

The long-lasting aim of our laboratory is the study of mouse polyomavirus life cycle (mPyV, small non – enveloped ds DNA virus). One part of our research is focused on events concerning the entry of mPyV into the host cell and its transport to the nucleus. Endocytic pathway misused by mPyV is not fully understood. It involves internalization of virions by smooth monopinocytic vesicles and entry into early endosomes. Further, after 1,5 – 3 h post infection, virus can be observed in recycling endosomes and endoplasmic reticulum. It is not clear where uncoating of mPyV takes place and how the virus genome is delivered into the nucleus.
The aims of this study were the following:

- Using plasmids, which encode dominant – negative mutant (DN mt) of caveolin-1 and Rab11 (marker of recycling endosomes), to determine the involvement of caveolin-1 and recycling endosomes respectively, in the productive pathway of mPyV
- To examine the role of proteasomes in productive infection
- Using the confocal microscopy to detect the time course of presence of VP1 (major capsid protein of mPyV) signal in the proximity of cell nucleus during mPyV infection

We found out, that overexpression of caveolin – 1 DN mt does not inhibit PyV infection. This supports our previous observations that mPyV can enter the cell in caveolae independent manner. Overexpression of Rab11 DN mt decreased mPyV infectivity. This indicates that recycling endosomes may be involved in mPyV productive infection. Using reversible proteasomal inhibitor MG-132, we observed the involvement of functional ubiquitin – proteasomal pathway in the early stage of mPyV life cycle: - Proteasomal inhibition caused a decrease of productive infection and this inhibition was dose dependent. - Inhibition effect was observed in the first 4 hours post infection, when mPyV entered host cells and traveled towards the nucleus. In contrast, proteasomal inhibition had no effect on the later stages of mPyV life cycle, which indicates that ubiquitin – proteasomal pathway is not involved in the early transcription. Surprisingly, inhibition effect of MG-132 could be overcome by higher multiplicity of infection.
While vast majority of virions reach perinuclear space approximately 3 hours post infection (signal of VP1 can be seen in endoplasmic reticulum or in recycling endosomes), we detected signal of VP1 in the close proximity of the nuclear membrane and also in the nucleus very soon after infection (1 hour). These results suggest, that a subpopulation of PyV can exploit a quick, alternative pathway for its trafficking towards the cell nucleus.

Key words: polyomavirus, virus entry, caveolae independent endocytosis, recycling endosomes, ubiquitin – proteasomal degradation, nuclear entry

Kľúčové slová: polyomavírus, vstup vírusu do buniek, endocytóza nezávislá na kaveolách, recyklujúce endozómy, ubiquitín – proteazomálna degradácia, vstup vírusu do jadra