Roles of cytoskeleton in mouse polyomavirus trafficking

ABSTRACT:

Mouse polyomavirus (mPyV) is small non-enveloped DNA virus. Its endocytic pathway is studied for a potential utilisation of polyomaviral virus-like particles in gene therapy and/or immunotherapy. mPyV enter cells by internalisation into smooth monopinocytic vesicles. During it´s journey through the cell, it pass through early endosomes, and at the time 3 hours post infection, it is localised in endoplasmic reticulum and recycling endosomes. Many aspects of mPyV trafficking and nuclear entry are not clear yet.

Time-lapse live imaging fluorescence confocal microscopy was used to describe the mouse polyomavirus intracellular movements. For these studies, we utilised mPyV fluorophore-labeled virions and cells expressing GFP-tagged g-actin or alpha-tubulin. Some virion-loaded vesicles were seen to move with actin organised into dynamic structures. Some of these structures resembled actin comets created by Listeria or vaccinia virus. At the same time post infection (40-60 min post infection), movement of the virion loaded vesicles along mirotubules was observed suggesting the simultaneous involvement of actin and tubulin during mPyV trafficking. Dynamitin, a dominant negative inhibitor of dynein-dynactin function reduced mPyV infection. Taken together with the known fact that disruption of microtubules by nocodazole treatment inhibits viral infection, it suggests an essential role for microtubule-dynein transport of mPyV during productive infection. To evaluate the role of actin during mPyV infection, we used some drugs affecting its dynamics. While actin stabilisation caused by jasplakinolide decreased infectivity, actin depolymerisation caused by latrunculin A increased infectivity of mPyV. These data sugest that while intact tubulin is essential for productive infection, actin rather plays a role in host cell defence.

Given the importace of microtubule-dynein dependent transport of mPyV, we intended to define the time point of the single virion docking at the nuclear membrane. It is known that viruses using molecular motor dynein for their direct journey to the perinuclear area are capable to reach the nucleus in 30-60 minutes post infection. Using live cell imaging, we observed single virions at close proximity to the nuclear membrane as soon as 30 minutes post infection while the majority of viral particles were spread in endosomal compartments in the cytoplasm of infected cells. Surprisingly, we observed the virion crossing the nuclear membrane at the time 45 minutes post infection. It suggests that the subpopulation of virions can exploit the faster pathway for their trafficking toward the nucleus.

For studies of individual steps of human BK polyomavirus infection, the constructions of expression plasmids for production of BKV structural proteins, VP1 or VP2 in mamalian cells were prepared.

Key words: mouse polyomavirus, virus trafficking, microtubules, actin microfilaments, dynein, nuclear entry, BK virus

Klíčová slova: myší polyomavirus, pohyb virionů, mikrotubuly, aktinová mikrofilamenta, dynein, vstup do jádra, virus BK