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Oncolytic H-1 Parvovirus Entry in Cancer Cells through Clathrin-Mediated Endocytosis

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Abstract : H-1 protoparvovirus (H-1PV) is a virus with inherent oncolytic and oncosuppressive activities while remaining non-pathogenic in humans. H-1PV was the first oncolytic parvovirus to undergo clinical testing. Results from trials in patients with glioblastoma or pancreatic carcinoma showed an excellent safety profile and first signs of efficacy. H-1PV infection is vastly dependent on cellular factors, from cell attachment and entry to viral replication and egress. Hence, we believe that the characterisation of the parvovirus life cycle would ultimately help further improve H-1PV clinical outcome. In the present study, we explored the entry pathway of H-1PV in cervical HeLa and glioma NCH125 cancer cell lines. Electron and confocal microscopy showed viral particles associated with clathrin-coated pits and vesicles, providing the first evidence that H-1PV cell entry occurs through clathrin-mediated endocytosis. Accordingly, we observed that by blocking clathrin-mediated endocytosis with hypertonic sucrose, chlorpromazine, or pitstop 2, H-1PV transduction was markedly decreased. Accordingly, siRNA-mediated knockdown of AP2M1, which retains a crucial role in clathrin-mediated endocytosis, verified the reliance of H-1PV on this route to enter HeLa and NCH125 cancer cells. By contrast, we found no evidence of viral entry through caveolae-mediated endocytosis. Indeed, pre-treatment of cells with nystatin or methyl-β-cyclodextrin, both inhibitors of caveolae-mediated endocytosis, did not affect viral transduction levels. Unexpectedly, siRNA-mediated knockdown of caveolin-1, the main driver of caveolae-mediated endocytosis, increased H-1PV transduction, suggesting caveolin-1 is a negative modulator of H-1PV infection. We also show that H-1PV entry is dependent on dynamin, a protein responsible for mediating the scission of vesicle neck and promoting further internalisation. Furthermore, since dynamin inhibition almost completely abolished H-1PV infection, makes it unlikely that H-1PV uses macropinocytosis as an alternative pathway to enter cells. After viral internalisation, H-1PV passes through early to late endosomes as observed by confocal microscopy. Inside these endocytic compartments, the acidic environment proved to be crucial for a productive infection. Inhibition of acidification of pH dramatically reduced H-1PV transduction. Besides, a fraction of H-1PV particles was observed inside LAMP1-positive lysosomes, most likely following a non-infectious route. To the author's best knowledge, this is the first study to characterise the cell entry pathways of H-1PV. Along these lines, this work will further contribute to understand H-1PV oncolytic properties as well as to improve its clinical potential in cancer virotherapy.

Keywords : clathrin-mediated endocytosis, H-1 parvovirus, oncolytic virus, virus entry
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