Abstract

Merkel cell polyomavirus (MCPyV) is a recently discovered human virus, having it´s genome often integrated in a genome of Merkel carcinoma cells. Although this type of carcinoma is not so usual, it is very aggressive and it´s incidence has been rising in last few years. It is not surprising that this virus is nowadays in the centre of scientific interest, as well as other pathogens and mechanisms affecting human life. Because the virus was discovered not so long ago, its research has been at the whole beginning. This diploma thesisaims to contribute to the study of this virus from the molecular-virology point of view. A neutralizing monoclonal antibody, type IgG2a, targeted against the main capsid protein of MCPyV, VP1, and recognizing its conformational epitote was prepared. This antibody was then used for a pilot study of VP1 VLPs MCPyV movement in mammalian cells. Results showed that the studied virus, at least particularly, utilizes caveolin-1-carrying vesicles for its movement in cells (colocalisation of VP1 VLPs and caveolin-1 was observedColocalisation with EEA1 marker of early endosomes, LamP2 marker of endolysosomal compartments or with BiP marker of endoplasmic reticulum was sporadic but significant. These preliminary results suggest that MCPyV might utilise an endocytic pathway leading through early and late endosomes, similar to that used by the mouse polyomavirus (MpyV) or the Simian virus 40. VP1 VLPs MCPyV entered cells considerably less effectively and slowly.in comparison with those of the mouse polyomavirus Next we created constructs for FLAG-fused minor proteins production and a construct for non-fused VP3 MCPyV production. Constructs producing VP2-FLAG and VP3-FLAG were used to study localization of minor proteins in the cell. The results showed that MCPyV minor proteins behavior is different from that of MPyV minor proteins. VP2 protein was detected in a small amount in the nucleus, most of it is then found in the cytoplasm close to the nucleus, while VP3 was detected only in the cytoplasm. Both, VP3 and VP3 have no striking affinity to intracellular membranes. No colocalizations with nuclear envelope was deteected and colocalisation with BiP marker of endoplasmatic reticulum was substantially less significant than that of the MPyV minor proteins. None of MCPyV minor proteins have a noticeable cytotoxic effect. While in the case of MPyV, histone H1 was translocated out of the nucleus and degraded (as a consequence of nuclear envelope demage), such effect was not observed in cells producing MCPyV VP2 or VP3.