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Ultrastructural investigation of human cytomegalovirus tegument protein UL71 and its role in secondary envelopment

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Background

The molecular mechanisms underlying the assembly of infectious virions of human cytomegalovirus (HCMV), an important human pathogen associated with complications in immunocompromised patients and the leading cause of birth defects after congenital infection, are still not well understood (Griffith et al., 2015). An important step of HCMV morphogenesis en route to infectious virions is a cytoplasmic envelopment process, referred to as secondary envelopment. Tegument protein pUL71, which is highly conserved among herpesviruses, is known to play an important role in the envelopment process (Schauflinger et al., 2011). Several functional motifs, mainly located in the conserved N-terminus of pUL71, have been characterized in detail (Read et al., 2019; Metzner, 2022), whereas the role of the non-conserved C terminus of pUL71 is mainly unclear. However, a tetralysine motif in the C terminal region of pUL71 has revealed a mechanistic involvement of this region in HCMV morphogenesis (Read et al., 2019). Dissecting the functions of pUL71 during secondary envelopment brings us further to unravel the molecular mechanism of secondary envelopment and could lead to the identification of new antiviral targets.

Methods

The role of the C-terminus of pUL71 was investigated by generation and characterization of a mutant virus (TB71insHA-299) expressing a truncated version of pUL71 fused to an HA epitope lacking the last 61 amino acids with the tetralysine motif and the MIM2 domain. A first phenotypic characterization of TB71insHA-299 after infection of human fibroblasts was performed by immunofluorescence microscopy (IF). For direct visualization of secondary envelopment, transmission electron microscopy (TEM) was carried out on human fibroblasts infected with TB71insHA 299 that were fixated by high-pressure freezing and freeze substitution at five days post infection and embedded in epoxy resin. Stages of secondary envelopment were analysed on ultrathin sections by TEM and compared with cells that were infected with wild-type virus or a previously published virus mutant carrying a mutation in the C-terminal tetralysine motif of pUL71 (Read et al., 2019). In addition, 3D analysis was performed on these samples by focused ion beam scanning electron microscopy (FIB-SEM) and scanning transmission electron microscopy (STEM) tomography (Villinger et al., 2014).

Results

In IF experiments, TB71insHA-299 infected cells showed an accumulation of viral particles at the periphery of the cytoplasmic viral assembly complex (cVAC) at large circular structures which is similar to a mutant lacking pUL71. Quantification of secondary envelopment stages by EM analysis of TB71insHA-299 infected cells revealed an impaired secondary envelopment compared to the wild-type virus but no further defect compared to the well characterized virus with a mutation in the tetralysine motif at the C terminus of pUL71 (Read et al., 2019). Furthermore, it was shown that the accumulation of viral particles at the periphery of the cVAC at large circular structures in TB71insHA 299 infected cells were multivesicular bodies (MVB) with viral capsids attached to the whole cytoplasmic face of the MVB membrane. Additionally, EM revealed abnormal budding of capsids at multiple budding sites (MBS). These ultrastructural differences and the impaired secondary envelopment are similar to a virus that is unable to express pUL71 (Schauflinger et al., 2011). In the virus with the mutated tetralysine motif, these ultrastructural differences could not be observed (Read et al., 2019).

Conclusion
In conclusion, the last 61 C terminal amino acids of pUL71, containing the tetralysine motif and especially the MIM2 domain of pUL71, seem to be of great importance for HCMV morphogenesis. This work demonstrates that additionally to the already characterized secondary envelopment defect caused by the mutation of the tetralysine motif, the other amino acids, most likely the MIM2 domain, carry additional functions for the formation of an intact cVAC and efficient secondary envelopment. This data argues for the controversially discussed involvement of the ESCRT machinery in HCMV morphogenesis and membrane remodelling.

Keywords:

HCMV, High-pressure freezing, virus morphogenesis

Reference:

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