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The targeting of US28 is dependent on GASP-1

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Background

The human cytomegalovirus (HCMV) encodes the seven transmembrane (7TM)/G protein-coupled receptor (GPCR) US28. US28 is able to endocytose and signal in a constitutive – i.e. ligand-independent – manner and is located predominantly in the membranes of intracellular organelles, especially late endosomes/lysosomes and multivesicular bodies (MVBs). It was suggested that the virions of HCMV may be budding into the membranes of these MVBs, where the viral receptors are then incorporated into the viral membranes during the final stages of virus assembly.

Methods

Protein-protein interaction between US28 and GASP-1 (G protein-coupled receptor-associated sorting protein-1) was investigated by GST-fusion protein-binding assay, MBP protein competition binding assay and co-immunoprecipitation. In HEK293 cells endogenously expressing GASP-1 the interaction between US28 and GASP-1 was disrupted by i) overexpression of dominant negative cGASP-1 or by ii) shRNA knock-down of endogenous GASP-1. The role of GASP-1 in the post-endocytic trafficking of US28 was analyzed by means of immunocytochemistry and biotin protection degradation assay.

Results

Here we show that GASP-1, which sorts many GPCRs to the lysosomes, also plays an important role in the post-endocytic targeting of US28. We find that disruption of the GASP-1/US28 interaction by either i) overexpression of dominant negative cGASP-1 or by ii) shRNA knock-down of endogenous GASP-1 is sufficient to alter the lysosomal targeting of US28 and also its post-endocytic degradation.

Conclusion

Our data suggest that the interaction of US28 and GASP-1 has important implications for the post-endocytic fate of US28.