Characterizing human cytomegalovirus-encoded G protein-coupled receptors UL33 and US28

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Abstract

Human cytomegalovirus (HCMV) is widespread in the human population. Infections are usually well controlled by the host immune system and therefore asymptomatic, after which the virus resides in a latent state to avoid elimination. Upon suppression or dysfunction of the immune system, virus reactivation can cause pathologies ranging from developmental defects, organ dysfunction and graft rejection to vascular and proliferative diseases. HCMV infection is associated with several malignancies, of which the link with glioblastoma is the best described, and HCMV is considered an oncomodulatory virus that aggravates the malignant potential of tumor cells.

Like all β- and γ-herpesviruses, HCMV encodes G protein-coupled receptors (GPCRs) derived from host chemokine receptors; UL33, UL78, US27 and US28. UL33 and US28 have been demonstrated to possess constitutive activity and promiscuous G protein coupling. These characteristics discriminate them from cellular receptor homologs but are shared with the oncogenic Kaposi's sarcoma-associated herpesvirus-encoded GPCR ORF74. Where human chemokine receptors depend on agonist binding for activation, triggering activation of one class of G proteins ($G\alpha_i$), the three viral GPCRs reside in active conformations, even in the absence of external stimulation, and couple to G proteins from multiple classes. In this way, viral GPCRs modulate cellular signalling networks and change the biology of host cells.

Although our current understanding of UL33, UL78 and US27 is rather limited, the cellular effects of US28 have been extensively studied. US28 can activate proliferative, proangiogenic and pro-inflammatory signalling networks resulting in tumor formation in animal models. Receptor protein and mRNA have furthermore been detected in glioblastoma tissue samples. Nonetheless, oncomodulatory properties of US28 have mainly been evaluated in disease-irrelevant cellular backgrounds, often not in context of viral infection.

In our first study we show that UL33, like US28, possesses oncomodulatory potential by constitutively activating multiple proliferative, angiogenic and inflammatory signaling pathways. Furthermore, *in vitro* spheroid growth and *in vivo* tumor growth were accelerated upon expression of this receptor in glioblastoma cells. Signaling and cellular effects stimulated by UL33 are mostly similar to US28, besides a few notable differences.

In our second study we report that the oncomodulatory properties of US28 in glioblastoma cells are driven by activation of the sphingosine kinase 1 (SK1)/sphingosine-1-phosphate receptor 1 ($S1P_1$) signalling axis. This route diverged into activation of AKT, cMYC, STAT3 and upregulation of CIP2A, and contains several feed-forward loops. Moreover, US28-mediated activation of STAT3 and increased SK1 and CIP2A abundance were confirmed in HCMV-Merlin infected glioblastoma cells.

In our third study we describe a growth defect in fibroblast cultures for HCMV Merlin mutant virus deficient of UL33, but not US28-deficient virus. UL33 facilitates virus spread mainly via the extracellular route, where both UL33 and US28 contribute to cell-associated dissemination of HCMV. Interestingly, UL33-deficient mutants of HCMV AD169, TB40/E and Towne strains have previously been reported to grow similar to their WT counterparts, which could suggest a strain-dependent role.

Altogether, the research described in this thesis expands our understanding of the pathological function of HCMV-encoded GPCRs UL33 and US28 in relation to oncomodulation as well as virus dissemination.