Summary

In this thesis, we present research into human cytomegalovirus (HCMV)-encoded G protein-coupled receptors (GPCRs) in glioblastoma multiforme (GBM). HCMV belongs to the family of beta-herpesviruses and codes for four known viral GPCRs: US28, UL33, US27 and UL78, of which US28 has been the most extensively researched. US28 is homologous to the human cytokine receptors CX3CL1, CCR1, and CCR2 (Couty & Gershengorn, 2005). US28 is able to bind to various ligands and signal both ligand dependently and independently to signaling pathways involved in proliferation, migration, inflammation, and angiogenesis (Maussang, Langemeijer, et al., 2009; Maussang, Vischer, Leurs, & Smit, 2009; Slinger et al., 2010). HCMV has been found in GBM patient material and has been correlated to tumor progression (Soroceanu et al., 2011). Moreover, patient survival increased upon treatment of GBM patients with Valganciclovir, a virus-inhibiting drug (Söderberg-Nauclér, Rahbar, & Stragliotto, 2013).

GBM is a malignant primary brain tumor with an average survival rate of 12 to 15 months (Hanif, Muzaffar, Perveen, Malhi, & Simjee, 2017). The tumor encompasses a variety of cell types, which often spread into the healthy tissue surrounding the tumor. This makes GBM challenging to treat, as complete removal is generally impossible. The remaining cells post-treatment are often resistant to therapy and able to proliferate, resulting in tumor relapse. These cells are also known as cancer stem cells (CSCs) and play a major role in the efficacy of GBM therapy. Researchers previously showed that HCMV is able to infect CSCs with a higher efficiency compared to GBM tumor cells, making these cells interesting as target for future drug development (Fiallos et al., 2014). The aim of this thesis is to gain knowledge about HCMV-encoded US28 and its role in the progression of GBM.

Aside from the HCMV and their viral GPCRs, other herpesviruses exist, each coding for their own specific viral GPCR. These viral GPCRs were found to be involved in different processes of tumor growth and development. In chapter II, we discuss these viral GPCRs, including ORF71, a GPCR encoded by the Kaposi Sarcoma virus (KSHV), BILF1, encoded by Epstein-Barr virus, and US28, UL33, and US27, encoded by HCMV.

To study the role of HCMV and US28 in a clinical relevant model, we develop a GBM 3D spheroid and in vivo mouse model in chapter III. We show that inducing US28 expression in these models, results in increased spheroid growth and tumor progression in animals. This is associated with an increase in the secretion of VEGF and IL-6. In chapter IV we develop US28-targeting nanobodies, which inhibit the constitutive activity of US28 as inverse agonists. We show that these US28 nanobodies are able to inhibit US28-mediated growth by 50% in our in vitro and in vivo models. Moreover, these nanobodies are able to reduce IL-6 levels in our animal models.

In chapter III and chapter IV, we show that HCMV infection in U251 GBM cells stimulate the development of neurospheres. Neurosphere formation is a characteristic of dedifferentiated cells and suggests that US28 might play a role in dedifferentiation of GBM cells. In chapter V we further look into this. We found that US28 affects the expression of epithelial and mesenchymal genes. The epithelial-mesenchymal (EMT) and mesenchymal-epithelial-transition (MET) play a role in cancer cell metastasis. However, no specific EMT or MET switch or epithelial/mesenchymal phenotype is induced as a result of US28 expression. However, induction of US28 does result in GBM dedifferentiation. In fact, US28 is able to signal towards
the major stemness regulators: Sox2 and OCT4, and stimulate the expression of CD133, a stem cell marker. These regulators of stemness are in turn under control of the Hippo pathway, in which YAP is a major transcription factor. These results suggest that US28 might play a role in cancer stem cell maintenance, possibly via the Hippo pathway, which in turn may affect tumor recurrence after therapy.

GBM is challenging to treat and eliminate, as these tumor cells are capable of evading the immune system (Razavi et al., 2016).