Summary

Glioblastoma (GBM) is the most common and highest grade malignant primary brain tumor in adults, with a very dismal prognosis. Despite aggressive treatment consisting of surgery, radiation therapy and chemotherapy, the survival benefit is extremely limited. Current therapies cannot tackle the invasive growth of this tumor and targeted therapies tested in clinical trials often fail due to tumor heterogeneity or limited blood-brain barrier (BBB) penetration and thus low bioavailability. Innovative treatment strategies are needed to address these issues. In this thesis we developed several adeno-associated virus (AAV) vector-based therapies targeting the brain. Furthermore, we looked at visualization of the interplay between GBM, platelets and extracellular vesicles (EVs) in the tumor microenvironment.

The first chapter introduces the subjects discussed in the other chapters of this thesis, in greater depth. Current therapies against GBM and their limitations are outlined, followed by intercellular communication via EVs, the involvement of platelets, and the use of preclinical models for therapeutic development. In addition, a detailed background of AAV is described, as well as its use as a gene therapy vector in clinical and preclinical studies. Literature related to the presented work and thesis aim is discussed.

In the second and third chapter, we used AAV as a delivery vehicle for soluble, secreted tumor necrosis factor-related apoptosis inducing ligand (sTRAIL) to the brain tumor milieu in the brain of GBM-bearing mice. As TRAIL has been shown to induce apoptosis in a variety of cancer cells, we used this as a model to study the delivery, expression and efficacy of AAV-mediated gene therapy against GBM.

Previous work from our laboratory has shown that different GBM cell lines show a varying amount of sensitivity to TRAIL-induced cell death and that this can be overcome by treatment with lanatoside C. In the second chapter, we developed an AAV.rh8 vector encoding sTRAIL and delivered it to the brain tumor environment via direct intracranial injection. Both a single injection of AAV vector at the tumor site or multiple injections around the tumor site showed some efficacy against GBM, and this effect was enhanced by systemic lanatoside C co-treatment. Moreover, lanatoside C could be used to sensitize TRAIL-resistant GBM cells in vivo, leading to a decrease in tumor growth.

GBM is a highly proliferative tumor with an invasive growth pattern, which poses a major obstacle in combating this disease. Intracranial injection of a vector has mostly local effects, incapable of reaching infiltrating tumor cells elsewhere in the brain. Due to the high vascularization of the brain, an intravenously (i.v.) administered vector could potentially have greater distribution compared to localized intracranial injections. However, access to the central nervous system (CNS) is...
tightly regulated by the BBB and many vectors are prevented from reaching the brain.

AAV serotype 9 (AAV9) can pass the BBB after i.v. administration, which we used in the third chapter as a vector for therapeutic transgene delivery to the brain. In addition, we looked at transcriptional targeting of transgene expression by using the neuron-specific enolase (NSE) promoter and compared it to the ubiquitous hybrid cytomegalovirus/chicken beta-actin (CBA) promoter. Transgene expression driven by the NSE promoter would be more restricted to the brain, while the CBA promoter is active in all tissues possibly leading to side effects after systemic administration of a vector. Using two different reporter genes, overall transgene expression was at least ten times lower with the NSE promoter compared to the CBA promoter. AAV9-mediated sTRAIL expression using either promoter lead to an increased survival of GBM-bearing mice, while there was a trend towards better survival using the CBA promoter compared to the NSE promoter.

In addition, we found that transgene expression of a reporter gene was increased in the brain of some mice compared to others. In the fourth chapter we describe that mouse gender influences transduction and transgene expression of i.v.-injected AAV9 vector, with increased expression levels in the brain and decreased expression levels in the liver of female mice compared to males.

During standard production of AAV in cell culture, we found that a portion of AAV vector is associated with EVs. Chapter five describes the use of EV-associated AAV as a gene therapy vector and incorporating a brain-targeting peptide on the surface of EVs, thereby enhancing transduction in the brain. Moreover, compared to “naked” AAV, EV-associated AAV was less sensitive to neutralizing antibodies, which are common in the general population and can completely inhibit AAV transduction of (target) cell populations. This provides evidence that EV-associated AAV could have potential as a novel gene therapy vector.

In recent years, it has become appreciated that EVs play an important role in intercellular communication. Both healthy cells and tumor cells produce EVs that can be taken up by other cells, thus transferring functional mRNA or protein from one cell to another. We made use of a switchable Cre-loxP reporter system in chapter six, to visualize the interactions between GBM cells, platelets and EVs. Transfer of Cre-containing EVs to reporter GBM cells in vitro caused some recombination, however, in vivo this process was much less efficient. Furthermore, Cre-containing platelets injected i.v. could home to the intracranial tumor and cause some recombination in reporter GBM cells. Similarly, some recombination was observed in GFAP-Cre expressing mice bearing syngeneic intracranial GBM reporter cells.

Finally, the seventh chapter discusses the work presented in this thesis and highlights some future perspectives and research avenues for AAV-mediated gene therapy in GBM.