Glioblastoma Multiforme (GBM) is the most common malignant brain tumor and is characterized by invasive and uncontrolled growth. Because of its invasive nature, it is not possible to completely remove GBM surgically and additional chemo- and radiation therapy have so far also not been able to eradicate GBM. Therefore, to develop new and better treatments, it is important to increase our understanding of the biological processes that play a role in the onset and progression of GBM.

Angiogenesis is an important driver of tumor progression, and significant efforts have been made to develop anti-angiogenesis therapies for cancer treatment. Among the tumors most characterized by extensive neovascularization are malignant gliomas, providing a rationale for anti-angiogenesis therapy. Among the many angiogenic factors involved in cancer, vascular endothelial growth factor (VEGF) has been shown to play a major role in pathological GBM angiogenesis and various strategies to target the VEGF pathway have been investigated. These include the use of monoclonal antibodies that bind VEGF, and small molecule inhibitors of the tyrosine kinases activated by the VEGF receptors. The proposed therapeutic mechanism behind these agents is a normalization of the tumor vasculature resulting in an improved delivery and efficacy of chemotherapeutics. Unfortunately, the therapeutic benefits of anti-angiogenic drugs have not been able to meet their high expectations in GBM. Inhibition of the VEGF pathway only produces temporary effects and is typically followed by tumor progression. Moreover, in preclinical studies of anti-angiogenic tumor treatment, an increase in tumor cell migration and metastasis was observed. The mechanisms of resistance to anti-angiogenic therapy are subject to ongoing investigations.

The aim of this thesis was to clarify the role of epigenetic and microRNA (miRNA) signaling in the regulation of gene expression in endothelial cells and GBM cells during angiogenesis.

Epigenetics refers to changes in gene expression or cellular phenotype caused by mechanisms that do not involve changes in the underlying nucleotide sequence. This explains how differentiated cells in an organism express different features whilst containing the same DNA sequence. For example, a neuron and a muscle cell have completely different appearance and functionality but both originate from the same primary precursor cell – the zygote – and carry the same DNA sequence.

MiRNAs are small, single-stranded, non-coding RNAs, that can block mRNA translation or can negatively regulate mRNA stability, and thereby play a central role in the regulation of gene expression. A single miRNA may target and regulate over 100 different mRNAs. As a result, miRNAs play a part in virtually all cellular processes such as proliferation, differentiation, apoptosis and migration. It is also becoming clear that deregulated miRNA expression is a common feature of human diseases, especially in specific forms of cancer. In chapter 2 we provide an overview of miRNAs that may be involved in GBM angiogenesis. In addition, potential therapeutic roles for miRNAs in GBM treatment are discussed.

In chapter 3 we demonstrate a correlation between the gradation of glioma and the expression of the oncogenic protein enhancer of zeste homolog 2 (EZH2). EZH2 is a histone-methyl-transferase that can alter the chromatin structure by adding a methyl group at specific locations. As a result DNA at these locations cannot be transcribed and
consequently various cellular processes are affected. EZH2 has an important functional role in embryogenesis in controlling (the initiation of) differentiation processes. However, in different forms of cancer EZH2 was also demonstrated to disable various tumor-suppressor genes and thus has a significant pathological role in tumor biology. We further show that increased EZH2 protein expression in glioma is caused - at least in part - by a decrease in miR-101 expression. In addition, we analyzed the relationship between EZH2 expression in GBM and proliferation, migration, invasion and angiogenesis. We also demonstrate that inhibition of EZH2 protein results in decreased proliferation, migration, invasion and angiogenesis in vitro and reduced tumor growth in vivo.

In chapter 4 it is shown that miR-101 expression is also reduced in GBM endothelial cells and that miR-101 expression in endothelial cells is suppressed by VEGF and conditioned medium derived from GBM cells. In accordance with the effect in GBM cells, the reduced miR-101 expression in endothelial cells results in increased EZH2 expression. We subsequently examined which genes in endothelial cells are regulated by VEGF and EZH2. Inhibition of EZH2 in endothelial cells by means of the experimental drug DZNep or by stimulating miR-101 resulted in reduced angiogenesis in vitro and reduced tumor growth in vivo.

In chapter 5, we show that the expression of miR-125b is also diminished in GBM endothelial cells. This increases the expression of the protein myc-associated zinc finger (MAZ). The decrease in miR-125b and subsequent increase in MAZ also occurred in endothelial cells in vitro that were stimulated with VEGF or conditioned medium derived from GBM cells. Since MAZ is a transcription factor for VEGF, the reduction of miR-125b in GBM endothelial cells results in an increase in VEGF and angiogenesis. Stimulation of miR-125b inhibits MAZ expression and results in reduced migration and angiogenesis in vitro.

Microvesicles - or exosomes - are nano-sized vesicles secreted by a wide range of cells and have been detected in virtually all body fluids such as plasma, cerebrospinal fluid and urine. The content of microvesicles differs from cell to cell and consists of various molecular constituents of the cells they originate from. This includes DNA, mRNA and miRNA. Neighboring or distant cells can take up microvesicles and absorb their contents. In this way, microvesicles can influence the behavior of the recipient cell. Microvesicle transfer has been shown to affect various cellular processes such as drug and immunoresponse and migration and invasion. As such, the role of microvesicles as a potential biomarker, as well as their role in cell-to-cell signaling, is actively being researched. In chapter 6 we show that endothelial cell derived microvesicles stimulate the angiogenic program in neighboring endothelial cells. We further demonstrate that the depletion of miR-214 in these microvesicles results in increased expression of ataxia telangiectasia mutated (ATM) and induction of senescence and suppression of the angiogenic program in recipient endothelial cells. These results are suggestive of an intricate microvesicle-mediated cross-talk interface at the vascular endothelium that prevents cell cycle arrest and regulates the angiogenic program - at least partly - via miR-214.

In chapter 7 we study the role of EZH2 and DAB2IP as a prognostic marker in medulloblastoma, a malignant brain tumor that occurs mainly in childhood. Current treatment modalities result in a 5-year survival rate of 80-90% in standard risk medulloblastoma patients. However, approximately 30% of patients remain incurable and current intensive treatment protocols cause significant adverse long-term effects such as endocrine dysfunction and reduced cognitive function. Currently, the staging for medulloblastoma between high risk and standard risk disease is based on clinical parameters and histological subtypes. However, it is suggested that inclusion of molecular markers in the risk stratification could improve survival and decrease treatment-related toxicity. We show that EZH2 represses DAB2IP and demonstrate that reduced DAB2IP expression correlates significantly with poor overall survival of medulloblastoma patients, independent of clinical variables such as age, metastatic stage and histology. Moreover, we show that ectopic DAB2IP expression enhances stress-induced apoptosis while reduced DAB2IP expression conveys resistance to irradiation-induced cell death in vitro.

In chapter 8 we discuss our results and reflect on future directions.