SUMMARY

In chapter 1 we provide a background on pediatric brain tumors in general, with more details on medulloblastoma, anaplastic ependymoma, anaplastic astrocytoma and glioblastoma multiforme. Besides considerable mortality, these tumors are associated with significant acute and long-term side effects of disease and treatment. To improve survival and quality of life, new, tumor-specific targets for therapy have to be explored. The main focus of this thesis was therefore to identify new treatment targets, based on laboratory data, for potential translation into in vivo studies and, ultimately, clinical trials.

Chapter 2 describes the role of AMPA-type glutamate receptors (AMPARs) in high-grade glioma (HGG). AMPARs were reported as potential targets for anti-tumor therapies with AMPAR inhibitors, since HGG cells were described to release large quantities of glutamate. This glutamate release into the microenvironment results in autocrine stimulation via AMPARs and excitotoxic cell death of normal neurons. However, in our study we show that these receptors are in fact down-regulated in HGG, compared to normal brain. Furthermore, using patch-clamp techniques, we demonstrate that HGG cells, expressing these receptors, do not show depolarization upon glutamate stimulation. Quantitative and functional down-regulation of AMPARs enables HGG cells to survive in a self-created high-glutamate environment, making AMPAR inhibitors less obvious as anti-tumor agents. These inhibitors however, might be of benefit in preventing glutamate-induced excitotoxicity in normal cells in the tumor microenvironment.

In chapter 3 we investigate the expression and inhibition of DNA-repair enzyme PARP1 in pediatric high-grade brain tumors. Inhibition of PARP1 was previously described to enhance sensitivity to chemo- and radiotherapy in other cancers, especially in tumors with defective double strand break (DSB) repair. In our study, PARP1-overexpression, as compared to normal brain, was found in patient material of medulloblastoma, anaplastic ependymoma and pediatric HGG, and this high expression correlated with lower overall survival. We found that medulloblastoma, anaplastic ependymoma and pediatric HGG cells could be sensitized to radiation by pretreatment with PARP1-inhibitor olaparib, resulting in the persistence of double strand breaks in these cells. These results are very promising and warrant further translation to in vivo and clinical studies in these tumors.

Chapter 4 focuses on a family of potential targets for treatment and radiosensitization in high-grade glioma: the ErbB family of growth factor receptors. Nowadays, EGFR and ErbB2 (HER2) are important treatment targets in multiple cancers. In silico EGFR- and ErbB2-overexpression in both adult and childhood HGG, as compared to normal brain, was validated in our study. Small molecule inhibitors and monoclonal antibodies directed against the ErbB family of growth factor receptors often only target one family member, which possibly allows
signaling via other, non-inhibited ErbB family members. To circumvent this, we investigated CI-1033, an irreversible pan-ErbB family small molecule tyrosine kinase inhibitor, as single drug, and in combination with radiotherapy in HGG cells. In two out of three HGG cell lines radiosensitization with CI-1033 pre-treatment was observed, although this was dependent on the pre-radiotherapy incubation schedule of the drug. In both these cell lines, reduction of Akt- and ERK-phosphorylation was detected, whereas no reduction of phosphorylated Akt and ERK was observed in the cell line that could not be sensitized to radiation. This study warrants further investigation on determinants of radiosensitization using CI-1033 in in vivo models of these cancers and translation to clinical studies.

In chapter 5 we used the Human Protein Atlas, a publicly available proteomics platform, on a multitude of normal and tumor tissue microarrays, to search in silico for highly-expressed malignant glioma-specific proteins, that are not, or only weakly, expressed in normal brain. We identified pre-B leukemia homeobox interacting protein 1 (PBXIP1) to be such a protein and validated its strong expression in high-grade astrocytoma and ependymoma patient material, whereas normal brain tissues were largely negative. Next, to investigate whether PBXIP1 could be a potential treatment target, we employed RNA interference using shRNA directed against PBXIP1 mRNA. PBXIP1-knockdown resulted in strong morphological changes in HGG cells, leading to a strong reduction of cell viability and motility. These results indicate that targeting PBXIP1 is an interesting option in the treatment of high-grade astrocytoma and ependymoma.

Chapter 6 is a study on SIRPα, a protein that is expressed in neurons and macrophages. SIRPα was previously found to be a tumor suppressor in other cancers. We examined SIRPα expression in medulloblastoma. In silico analysis and immunohistochemical studies revealed down-regulation of SIRPα by promoter hypermethylation and, possibly, by micro-RNA clusters miR-17/92, miR-106a/25 and miR-106b/363, overexpressed in MB. Targeting of epigenetic modulators with DNA methyltransferase, histone-deacetylase (HDAC) and -methyltransferase (HMT) inhibitors, resulted in strong SIRPα up-regulation and reduction of MB cell viability. Lentiviral inducible SIRPα overexpression did not affect MB cell viability. The mechanism of the anti-MB action of epigenetic therapy needs further investigation since our data indicate that this effect might be SIRPα independent.