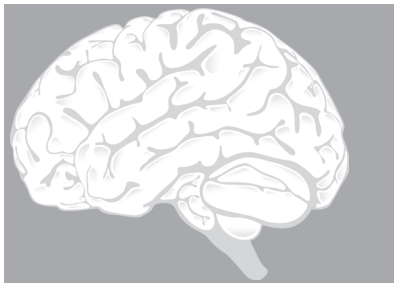
CHAPTER 8



Summary

Primary high-grade brain tumors pose a severe problem in both adults and children. Despite multimodality treatment consisting of surgery, radiotherapy, and chemotherapy, outcome remains poor, especially in patients with certain types of medulloblastoma and in those with high-grade gliomas (glioblastoma; GBM, diffuse intrinsic pontine glioma; DIPG). Resistance to therapy is one of the main obstacles that impairs treatment efficacy in these patients. Furthermore, patients who respond well to treatment often suffer from severe treatment-related side effects. Therefore, new treatment strategies are urgently needed for high-grade brain tumors. The aim of this thesis was to identify mechanisms underlying treatment resistance in high-grade brain tumors, to interfere with these mechanisms, and, thus, to improve currently applied treatment modalities in GBM, medulloblastoma, and DIPG.

In **chapter 2**, we explore mechanisms underlying temozolomide (TMZ) resistance in GBM using a model of acquired TMZ resistance consisting of three TMZ-sensitive wild type GBM cell lines (U87, Hs683, and LNZ308) and their six TMZ-resistant subclones. Gene expression profiling of these cell lines identified EFEMP1, an extracellular matrix protein, to be involved in TMZ resistance. Upregulation of EFEMP1 is associated with a TMZ-resistant phenotype, while downregulation of EFEMP1, using an siRNA, decreased cell survival following TMZ treatment. EFEMP1 can exert its effect via multiple signaling pathways, including the Notch pathway. Inhibition of the Notch pathway using the gamma-secretase inhibitor RO4929097 resulted in at least partial sensitization of GBM cells to TMZ, both *in vitro* and *in vivo*. Furthermore, we showed that EFEMP1 expression correlates with survival in patients treated with TMZ. These results show that EFEMP1 could be a potential target to overcome TMZ resistance in GBM.

Chapter 3 describes the usefulness and feasibility of integrative miRNA/mRNA regulatory network analysis using mirConnX to identify TMZ resistance factors in GBM. We further employed the TMZ resistance model of TMZ-sensitive and TMZ-resistant GBM cells in this chapter. miRNA profiles of these cell lines were obtained and integrated with these cells' mRNA profiles (obtained in chapter 2) using the network analysis tool mirConnX. We identified the plant homeodomain (PHD)-like finger 6 (PHF6) as a potential TMZ resistance factor in the TMZ-resistant cells. Analysis of PHF6 expression on both mRNA and protein level in GBM and normal tissue showed that PHF6 is overall highly expressed in GBM compared to normal tissue. To determine whether PHF6 could be functionally involved in TMZ resistance, we decreased PHF6 expression in three TMZ-resistant subclones using an siRNA against PHF6 and showed that PHF6 downregulation in combination with TMZ treatment significantly enhanced cell kill in two of these subclones. Altogether, we demonstrated the usefulness and feasibility of miRNA/mRNA network analysis using mirConnX to explore for TMZ resistance factors in TMZ-resistant GBM cells.

In **chapter 4**, the TMZ resistance model of TMZ-sensitive and TMZ-resistant GBM cell lines is used one more time to identify therapeutic agents that could sensitize GBM cells

to TMZ. Through drug screening, we identified hydroxyurea (HU) as a potential TMZ sensitizer. Further evaluation of HU in both newly diagnosed primary GBM cells and recurrent, TMZ-resistant, GBM cells *in vitro* and *in vivo* showed that HU could enhance the TMZ effect, irrespective of the MGMT promoter methylation status of the cells. HU may exert its effect by targeting ribonucleotide reductase subunit M2 (RRM2), an enzyme involved in nucleotide metabolism, thereby limiting *de novo* DNA synthesis. Knockdown of RRM2 in combination with TMZ enhanced the TMZ effect in GBM cells, similar as observed for HU combined with TMZ. Previous studies evaluating HU as a therapeutic agent for malignant gliomas have shown limited efficacy of the drug. This is the first demonstration that HU combined with TMZ has potential as adjuvant therapy for GBM patients.

Chapter 5 focuses on the flavonoid quercetin as potential radiosensitizer for medulloblastoma. Quercetin was identified to improve the efficacy of radiation in DAOY cells by screening of a small molecule library consisting of 960 chemical compounds. We further evaluated the radiosensitizing potential of quercetin in D283 and D458 cells and showed that quercetin could sensitize these cells to radiation at low micromolar concentrations, as determined in cell viability and clonogenic assays. Moreover, quercetin did not affect the proliferation of neural precursor cells or normal human fibroblasts. The radiosensitizing effect of quercetin was not observed in primary medulloblastoma cells, probably due to the high intrinsic radiosensitivity of these cells, and the subsequent lack of a window in which quercetin can exert its effect. *In vivo* analysis of quercetin in a D283 xenograft model shows that quercetin in combination with radiation can prolong the survival of mice compared to treatment with quercetin or radiation alone. Since medulloblastomas are classified into four distinct molecular subgroups, we determined whether the effect of quercetin on the radiation response was subgroup-dependent. DAOY cells were identified as a Sonic Hedgehoc (SHH) medulloblastoma, while D283 and D458 belong to the group 3 medulloblastomas. Therefore, this might indicate a subgroup-independent radiosensitizing effect of quercetin in medulloblastoma. These results indicate that quercetin as radiosensitizer could be a therapeutic venue for medulloblastoma patients.

In **chapter 6** we determined whether the WEE1 kinase inhibitor MK-1775 could enhance the radiation response in DIPG. Radiotherapy is the standard treatment for patients with DIPG since no therapeutic agent has shown to be effective in this malignant brain tumor type. WEE1 kinase is one of the main gatekeepers of the G2 cell cycle checkpoint in which radiation-induced DNA damage can be repaired. WEE1 kinase was identified to be highly expressed in DIPG tissue compared to non-neoplastic brain tissue. Inhibition of WEE1 kinase in DIPG cells by the clinically relevant inhibitor MK-1775 resulted in decreased irradiation-induced WEE1-mediated phosphorylation of CDC2 and reduced G2-M arrest. Subsequently, this resulted in decreased viability of these DIPG cells. In addition, we showed that MK-1775 could enhance the radiation response in an E98-Fluc-mCherry mousse xenograft model.

This indicates that WEE1 inhibition in combination with radiotherapy is an interesting option for treatment of DIPG.

In **chapter 7**, the work described in this thesis is discussed and future directions are reflected upon.