CHAPTER 1

General introduction
GENERAL INTRODUCTION

Primary high-grade brain tumors pose a severe problem in both adults and children. The term “brain tumors” comprises a wide variety of tumor (sub-)types which differ in their location within the brain, their cellular origin, developmental timing, histological phenotype, biological behavior, and molecular phenotype. Even within a specific brain tumor subtype, we can distinguish different subgroups based on morphology and molecular phenotype. The heterogeneous appearance and location make these tumor entities difficult to treat. Also, intrinsic and acquired resistance to treatment represents a major clinical challenge in these patients. As a result, the prognosis for patients with the most aggressive variants of these tumors is extremely poor. Furthermore, treatment modalities can cause severe side- and late effects, especially in children, which further hinders treatment of these patients. In this thesis, we aim to elucidate resistance mechanisms to conventional therapies, in order to be able to interfere with these mechanisms, and test novel therapeutic agents in preclinical models to improve treatment efficacy.

HIGH-GRADE BRAIN TUMORS

Central nervous system (CNS) tumors are still classified and graded according to the 2007 WHO classification and grading scheme, although a new classification system incorporating recent molecular genetic information is being developed. More than 100 types of CNS tumors are described in this scheme, including 59 tumor (sub-) types of neuroepithelial tissue. The frequency, location in the brain, and most common types of brain tumors differ in adults and children. Glioblastoma (GBM) is the most commonly diagnosed malignant tumor type in adults, while medulloblastomas arising in the cerebellum are predominantly found in children. The different spectrum of brain tumors seen in adults and children could be explained by the influence of distinct precursor and mature cells on tumor development within the developing and mature brain. In addition, the distinct microenvironments could also play a role in this process. Here, we focus on glioblastoma, the most common and most malignant (WHO grade IV) brain tumor type found in adults, medulloblastoma (WHO grade IV), the most common high-grade brain tumor type found in children, and diffuse intrinsic pontine glioma (DIPG), the deadliest brain tumor in children.

Glioblastoma

GBM is the most frequently occurring, and most aggressive primary brain tumor in adults. Patients diagnosed with this disease have an extremely poor prognosis with a median survival of approximately 14 months. These tumors are generally located in the cerebral hemispheres, and show an infiltrative growth pattern. GBMs are histologically characterized by atypia, mitosis, endothelial proliferation, and areas of necrotic tissue surrounded by anaplastic cells. These tumors were thought to originate
from glial cells, which are non-neuronal cells that provide support and protection to neurons. However, recent studies suggest that GBMs can also arise from neural stem cells, progenitor cells, or even from other differentiated cells such as neurons. Therefore, the exact cellular origin of GBMs remains unclear.

Several genetic aberrations are observed frequently in GBM, including amplifications of PDGFR, EGFR, MET, MDM2, MDM4, and CDK4, deletions of PTEN, CDKN2A/CDKN2B, and NF1 or mutations in EGFR, PI3K, MET, NF1, and TP53. These aberrations result in deregulation of three key signaling pathways in glioblastoma, namely RB (retinoblastoma), p53, and RTK (receptor tyrosine kinase)/RAS/PI(3)K (phosphoinositide 3-kinase) pathways, which have been suggested to be obligatory events in probably all GBMs. More recent studies by The Cancer Genome Atlas (TCGA) have provided a comprehensive overview of somatic alterations in GBM based on genomic, transcriptomic, epigenomic, and targeted proteomic profiling of GBMs. These studies have identified four distinct GBM subtypes based on their molecular phenotypes. These subtypes, classical, pro-neural, neural, and mesenchymal, are characterized by aberrations and differences in gene expression of several marker genes. The classical subtype is characterized by aberrations in EGFR, mostly amplification of the gene. Abnormalities in PDGFRA and mutations in IDH1 and TP53 are associated with a pro-neural subtype, while mesenchymal subtype GBMs are characterized by a high frequency of mutations/deletions in NF1 and low NF1 mRNA expression. The neural subtype expresses neural markers, and the gene expression profile of these GBMs is most similar to normal brain tissue. These subtypes have been shown to respond differently to aggressive therapy, where the classical and mesenchymal subtype show a survival advantage after a more intense therapy regimen. However, the beneficial effect of aggressive treatment is not seen in patients with a pro-neural subtype GBM. Subclassification of GBMs may ultimately be used to direct treatment strategies.

In contrast to its frequency in adults, GBM is far less common in children and accounts for approximately 3% of all pediatric CNS tumors. Outcome for children with GBM is quite dismal, with 5-year survival rates ranging from 5 to 15%. Similarly as in adults, GBMs in children (excluding DIPGs) are most commonly found in the cerebral hemispheres. Furthermore, these tumors are generally histologically indistinguishable from adult GBM, displaying similar characteristics such as an infiltrative growth pattern, high cellularity, nuclear atypia, high mitotic activity, and microvascular proliferation. On the other hand, the molecular phenotypes of pediatric GBMs seem to substantially differ from their adult counterparts. Molecular profiling of pediatric GBMs has uncovered genetic alterations underlying these tumors, which show key biological differences with adult GBMs. For example, chromosomal abnormalities commonly observed in adult GBM, such as gain of chromosome 7 and loss of chromosome 10q, are less frequent in pediatric GBMs. In addition, the most common genetic aberration found in adult GBM, EGFR amplification, is less frequently observed in pediatric GBM, although these tumors often show EGFR overexpression. In contrast, pediatric GBMs
display molecular aberrations not commonly found in adult GBMs. For example, driver mutations in histone H3.3 and chromatin remodeling genes are almost exclusively detected in pediatric GBMs. The findings obtained from molecular profiling studies indicate that pediatric and adult GBMs should be considered two different entities and, therefore, one should be careful with extrapolation of data from adult GBM to pediatric GBM.

**Medulloblastoma**

Primary brain tumors are the second most common type of childhood cancer, with medulloblastoma being the most common malignant brain tumor type, accounting for approximately 20% of all intracranial tumors. Medulloblastoma is classified as a highly aggressive WHO-grade IV embryonal tumor, which is located in the cerebellum. Currently, patients are stratified in high- and standard-risk groups, based on the child’s age, the extent of surgical resection, and disease spread. Furthermore, medulloblastomas have been classified, based on histology, as desmoplastic/nodular, classic, large cell, anaplastic, and medulloblastoma with extensive nodularity (MBEN). The histopathological features of medulloblastoma have been employed for risk stratification and outcome prediction. More recently, it has been shown that medulloblastoma comprises four distinct molecular subtypes based on gene expression profiles: sonic hedgehog (SHH), WNT, group 3, and group 4. These groups have different clinical outcomes, histology (SHH and WNT), age groups, and gender biases.

Medulloblastomas belonging to the WNT subgroup are characterized by aberrations in the WNT signaling pathway and currently have the best prognosis with a 5-year overall survival rate of approximately 95%. WNT subtype medulloblastomas form the least common subtype with a frequency of 10%. The SHH subgroup has a frequency of 30% and, like the WNT tumors, a defined aberrant oncogenic pathway is involved in tumorigenesis: the sonic hedgehog-signaling pathway. SHH tumors have a 5-year overall survival rate of 75%. The oncogenic mechanisms that play a role in the other two subgroups are less clear, and these are therefore designated group 3 and group 4. Group 3 tumors are marked by high-level amplification of MYC proto-oncogene, and have a frequency of 25%. Group 4 occurs in approximately 35% of the cases, and often shows amplification of MYCN and CDK6. Patients with a group 4 medulloblastomas have an intermediate prognosis (5-year overall survival of 75%) while patients with a group 3 medulloblastoma have the worst prognosis of all subtypes (5-year overall survival of 50%).

Medulloblastomas are thought to arise from progenitor and precursor cells of the dorsal brain stem and cerebellum, respectively. Recently, cellular origins for three of four subgroups have been specified (Fig. 1). Lower rhombic lip progenitors (LRPs) have been implicated as the cell of origin for WNT medulloblastomas. SHH medulloblastomas arise in cerebellar granule neuron precursors (CGNPs) of the
cerebellar external granule cell layer (EGL) and of cochlear nuclei of the brainstem but also in neural stem cells (NSCs) in the subventricular zone. CGNPs in the EGL and NSCs are responsible for the generation of group 3 medulloblastomas. The cellular origin of group 4 tumors remains unclear.

**Diffuse intrinsic pontine glioma**

High-grade gliomas represent 15%-20% of all pediatric CNS tumors and a proportion of these tumors are diffuse brainstem gliomas. The majority of these diffusely
Growing tumors arise in the pons of the brain, a very delicate area in the brainstem, which regulates many critical functions including breathing and blood pressure. These tumors are called diffuse intrinsic pontine gliomas (DIPG) and patients with these tumors have an extremely poor prognosis. It is the deadliest brain tumor type in children and less than 10% of DIPG patients survive longer than two years after diagnosis.

Until the previous decade, not much was known about the biology of these tumors due to the rarity of the disease and a lack of patient material to study. In the last few years, several studies have examined the genetic make-up of DIPG, and looked into the possible cell of origin, driver mutations and oncogenic aberrations present in these tumors. These studies implicate that altered gene expression during development plays a role in DIPG pathogenesis. Recently identified mutations in histone H3.3 and H3.1, present in nearly 80% of DIPGs, could be involved in aberrant gene regulation in DIPG. These mutations encode p.Lys27Met (H3K27M) substitutions, changing the methylation status of histone 3, which subsequently alters gene expression. DNA structural aberrations found in DIPG include gain of chromosome 1q, 7p and 7q and loss of 10q, the last associated with loss of the tumor suppressor gene PTEN. Furthermore, aberrations in INK4A/ARF, PDGFRA, EGFR, and IRS2 suggest the importance of the PI3K pathway in DIPG. Alterations in TP53, MDM4, MYCN, and CDKN2A, amongst others, have also been described in DIPG. A previously not identified aberration in cancer concerns activating mutations in the ACVR1 gene, which are present in 20% of DIPG tumors. This could represent a new target for therapeutic intervention. In addition, as for other brain tumor types as described above, a recent paper suggests that DIPG consists of three distinct molecular subtypes: H3K27M, silent, and MYCN. The H3K27M subtype is highly mutated in histone H3.3 of H3.1 and has a highly unstable genome. DIPGs of the silent subtype have silent genomes and a lower mutation rate than DIPGs in the other subtypes. The MYCN subtype is characterized by MYCN amplification, hypermethylation, and high-grade histology. This subclassification of DIPG could help in guiding future therapy selection for DIPG patients.

**TREATMENT**

Although treatment strategies of the various brain tumor types differ, conventional treatment regimens consist of a combination of surgery, radiotherapy, and chemotherapy. In some tumors, such as medulloblastomas, these treatment regimens have resulted in improved survival rates, however, no improvement of survival has been realized in three decades for DIPG. Increased understanding of mechanisms underlying tumorigenesis of brain tumors and identification of key oncogenic signaling pathways in brain tumors that can be targeted, has moved research towards the development of targeted therapies. However, none of the targeted therapies tested in clinical trials has been implemented in first-line treatment regimens of high-grade brain tumors thus far.
Glioblastoma

Treatment of GBM consists of the conventional treatment modalities, surgery, radiotherapy, and chemotherapy. Because of the infiltrative nature of GBMs it is impossible to resect the tumor completely, therefore, patients are subsequently treated with adjuvant radio- and chemotherapy. The chemotherapeutic agent temozolomide (TMZ) has been added to the treatment regimen after demonstration that concomitant and adjuvant TMZ with radiation significantly improves median survival of GBM patients by 2.5 months\textsuperscript{6,7}. TMZ is an alkylating agent, which hydrolyzes to the active component 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC) at physiological pH\textsuperscript{62}. In the cell, MTIC converts to an inactive metabolite while methylating the DNA at specific nucleotides (Fig. 2). The most commonly methylated nucleotides are the N-7 and O-3 positions of guanine and adenine, respectively\textsuperscript{62,63}. The most cytotoxic but less common (approximately 5%) methyl-adduct induced by TMZ metabolism is on the O-6 position of guanine\textsuperscript{64,65}. During DNA replication, this guanine is mismatched with a thymidine instead of a cytosine, which is recognized by the mismatch repair (MMR) machinery of the cell and removes the thymidine. However, a thymidine is again incorporated opposed to the methylated guanine, resulting in futile cycling of the MMR system, which eventually leads to the induction of double strand breaks and cell death\textsuperscript{66–70}. An important characteristic of TMZ that made it promising for treatment of GBM, is its capacity to pass the blood-brain barrier (BBB), an important hurdle to take in the treatment of brain tumors\textsuperscript{71,72}. The BBB separates the brain from the peripheral circulation and is required to defend the brain against circulating toxins and harmful chemicals. This barrier consists of a dense network of blood capillaries in which the endothelial cells are joined together by tight junctions, impairing the uptake of chemicals from the peripheral circulation. This is a major obstacle for drug delivery to brain tumors. In addition, drug efflux pumps present on the endothelial surface further hamper the delivery of drugs to the brain. These are usually also expressed on tumor cells, forming an additional barrier for drugs to enter these cells.

Similar as for adult GBM, treatment of pediatric glioblastoma consists of a combination of surgery, radiotherapy, and chemotherapy. Since clear tumor boundaries are lacking in GBM, an attempt is made to remove as much tumor as possible without causing any additional neurological deficit. Subsequently, pediatric GBM patients (>3 years of age) are treated with a combination of radio- and chemotherapy. The additional benefit of chemotherapy for the treatment of pediatric GBM has been shown in a randomized clinical trial comparing radiotherapy alone versus radiotherapy plus lomustine, prednisone, and vincristine (PCV)\textsuperscript{73} and in a subsequent trial comparing the PCV regimen to a new experimental ‘8-drugs-in-1-day’ regimen\textsuperscript{74}. Since TMZ has been shown to significantly improve survival of adult GBM patients, its efficacy has also been tested in pediatric GBMs\textsuperscript{75}. However, no beneficial effect of TMZ was observed compared to conventional chemotherapy. Nonetheless, the improved tolerability to TMZ over other
chemotherapeutics and the ability to add other agents to this treatment regimen has adopted TMZ as one of the commonly applied therapies for pediatric GBM\textsuperscript{18}.

Knowledge on the biology of GBM is expanding significantly, and has provided new insights into potential novel treatment targets in GBM. Goal is to identify agents that have cytotoxic effects by themselves, or in combination, or to improve the efficacy of current treatment modalities. Since more and more molecular aberrations underlying GBM tumorigenesis and several key oncogenic pathways in GBM, are identified, research is moving towards the development of targeted therapies for GBM. Targeting specific proteins important in GBM progression may help in specifically targeting tumor cells while leaving healthy cells unharmed, thereby reducing treatment-related side-effects. Numerous clinical trials examining the efficacy of targeted agents have been conducted, are ongoing, and will be started in the future\textsuperscript{76-79}. So far however, none of these agents tested in trial setting have been implemented in the treatment schedule of GBM.

**Medulloblastoma**

Patients diagnosed with medulloblastoma first undergo surgery to resect the tumor to the maximum extent feasible. Surgery is followed by craniospinal radiation and chemotherapy\textsuperscript{23,80-82}. Based on the age of the child, extent of surgical resection, and
spread of the disease, a patient is grouped in either the standard-risk group or the high-risk group. Patients in the standard-risk group are treated with reduced-dose craniospinal radiation (23.4 Gray (Gy)) whereas high-risk patients are treated with a higher dose (36 Gy). Adjuvant chemotherapy consists of different combinations of chemotherapeutic agents, which usually include cisplatin, lomustine, vincristine, cyclophosphamide, and etoposide, and has significantly reduced the risk of tumor recurrence. Children younger than three years of age are not treated with radiation due to the associated devastating neurocognitive sequelae. In these children, adjuvant chemotherapy delays or avoids the need for radiotherapy. The current treatment regimen has significantly improved the overall survival of children with medulloblastoma and cure rates are nowadays >80% and >60% for patients with standard- and high-risk medulloblastoma, respectively.

The recent classification of medulloblastoma in distinct molecular subtypes will facilitate the stratification of patients to treatment options, especially when novel agents targeting the specific aberrations in the four different subgroups can be applied. One such agent is vismodegib, which targets the SHH signaling pathway and is currently tested in clinical trials for treatment of patients with recurrent/refractory medulloblastoma.

Diffuse intrinsic pontine glioma

Therapeutic options for the treatment of DIPG are limited. Surgery in these patients is not possible due to the delicate location of the tumor. Therefore, the standard of care for DIPG patients is focal radiotherapy to a maximum dose of 54-60 Gy. Radiotherapy has been shown to provide transient neurologic improvement in about a third of the patients and appears to control tumor growth for a short period of time, however, progression of the disease is inevitable. Many chemotherapeutic agents have been tested in clinical trials but none have significantly improved outcome beyond that achieved with the current standard of care.

Clinical Challenges

Failure of treatment remains a central problem in the management of malignant brain tumors, and especially in GBM and in DIPG patients treatment failure is currently unavoidable. Incomplete surgical removal, inefficient delivery of agents to the tumor, ineffective concentrations of the therapeutic agent at the tumor site, and intrinsic- and acquired resistance to the treatment modalities all have their adverse influence on treatment efficacy. In contrast, survival rates have improved significantly in children with medulloblastoma showing efficacy of current therapy strategies in this tumor type, especially in patients belonging to the standard-risk group (see risk description above). However, treatment still fails in approximately 30% of mainly high-risk medulloblastoma patients. Moreover, therapy causes severe long-term side-effects in survivors of medulloblastoma, which has a tremendous impact on quality of life. Therefore,
current treatment strategies need to be improved to increase treatment efficacy and decrease treatment-related side effects.

**Glioblastoma**

Besides incomplete tumor resection, intrinsic and acquired resistance to treatment present key obstacles in the management of GBM. Resistance to both radiation and temozolomide contributes to the poor prognosis of adult and pediatric GBM patients. In this thesis, we will focus on the mechanisms that could cause resistance to TMZ. The most well-studied TMZ resistance mechanism is the activity of the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT)\textsuperscript{96–98}. This enzyme removes the methyl group from the O6 position of guanine, the most cytotoxic adduct, thereby also inactivating itself\textsuperscript{99,100}. MGMT expression seems to be regulated by methylation of its promoter site and tumors with a methylated promoter appear to be more sensitive to TMZ than tumors with an unmethylated MGMT promoter\textsuperscript{101–103}. It has been shown that GBM patients with a methylated MGMT promoter have a better prognosis than patients with an unmethylated MGMT promoter\textsuperscript{104,105}. Therefore, methylation of the MGMT promoter has been used as a predictive marker for response to TMZ treatment\textsuperscript{98}. However, there are still patients who have low MGMT activity but show resistance to TMZ\textsuperscript{96,97}. This indicates that other mechanisms play a role in the resistance to TMZ. Deficiency in MMR, even as activity of the base excision repair (BER) machinery, which is involved in repair of the adducts other than O6-methylguanine, render GBM cells resistant to TMZ\textsuperscript{106–110}. Much effort is being put into the elucidation of mechanisms involved in TMZ resistance. Many studies have contributed to clarify the puzzle of TMZ resistance and show the involvement of various signaling pathways and molecules\textsuperscript{111–121}. One of the aims of this thesis is to contribute to the solution of this puzzle and to elucidate mechanisms underlying TMZ resistance.

**Medulloblastoma**

Outcome for medulloblastoma patients has remarkably improved during the last decades, however, treatment failure still occurs in approximately 30% of patients. Another striking problem in the management of medulloblastoma is the long-term treatment-related side-effects, which severely impact quality of life of these patients\textsuperscript{95}. Especially craniospinal radiotherapy and its effect on surrounding healthy tissue are responsible for these treatment-related side-effects. Many medulloblastoma survivors develop neurocognitive deficits\textsuperscript{122–131}. The extent of these side-effects depends on the total dose of radiation, the total brain volume irradiated, and the age of the patient. Furthermore, individual susceptibility and genetic factors could also play a role in the severity of the side-effects of radiotherapy.

There is a need to improve the efficacy of radiation or to lower the dose of radiation, especially in the very young patients. Improving currently used techniques
for radiotherapy in order to reduce the primary site target volume and as such to limit the exposure of healthy tissue to the harmful radiation, is an option to reduce harmful side-effects. In order to improve the efficacy of the therapy, the use of agents that sensitize tumor cells to radiation and also show limited toxicity in healthy surrounding cells, is proposed. Expanding our knowledge of medulloblastoma biology and of the recently identified molecular medulloblastoma subtypes will help in the development of such therapeutic agents that sensitize tumor cells to conventional therapies.

**Diffuse intrinsic pontine glioma**

As mentioned previously, therapeutic options for DIPG are scarce. Current standard of care consists of radiotherapy but this treatment modality shows only limited effects for a short period of time in a minority of patients. Many therapeutic agents have been tested in clinical trials but have not resulted in an improved outcome of DIPG patients\(^{90,92,93}\). Lack of knowledge of the biology of DIPG due to limited availability of material has impeded the development of more targeted therapies. However, knowledge is now rapidly expanding and more and more DIPG samples are becoming available to study underlying tumorigenesis of DIPG\(^{132–134}\). This allows for the identification of therapeutic targets that could be vital for DIPG progression. Interfering with these targets could help to inhibit tumor growth by itself or help in sensitizing tumors to conventional therapy.

The lack of efficacy of therapeutic agents in DIPG could also be caused by inadequate delivery of therapeutic agents to the tumor site. As mentioned above, the BBB is a major obstacle for efficient drug delivery to the brain and once in the brain, diffusion of drugs through the brain parenchyma is limited. The degree of drug penetration in DIPG tumors remains unknown due to a lack of in vivo DIPG models\(^{47}\). Therefore, it is necessary to study drug uptake in DIPG tumors and to improve the delivery of drugs to the brainstem. One way to improve delivery of therapeutic agents to the tumor might be convection-enhanced delivery, in which agents are delivered under continuous low-pressure directly into the tumor via a catheter\(^{47,135}\). In addition, disruption of the BBB and inhibition of drug efflux pumps on the surface of endothelial and tumor cells might also improve drug delivery to the brain. The use of drugs that have a natural capacity to efficiently penetrate the DIPG tumor would overcome all these issues. However, this would also increase the likelihood of neurotoxicity resulting from brain uptake of these drugs.

**PRECLINICAL BRAIN TUMOR MODELS**

Resistance to treatment in brain tumors is a major clinical problem and improvement of current treatment strategies of GBM, medulloblastoma, and DIPG is urgently needed. Relevant and reliable preclinical models are essential to be able to elucidate mechanisms underlying treatment resistance, to interfere with these mechanisms and
to develop novel treatment strategies. Both in vitro and in vivo models are being used to identify resistance mechanisms and to test novel therapeutics. In addition, preclinical models can be used to identify predictive biomarkers for treatment response that would help in the development of novel therapeutic strategies.

In vitro models
Cell lines of human brain tumors are often used to study brain tumor biology and to test novel therapeutic agents. Established cell lines such as U87 and U251 are frequently used for these purposes. However, long-term culture of these cell lines in vitro has lead to a high level of selection and adaptation of these cells to the culture conditions\textsuperscript{136}. Primary cell cultures isolated from brain tumor samples are more likely to recapitulate the characteristics of the original tumor. A lot of effort is being put into the optimization of culture protocols of these primary cultures to retain the features of the original tumor\textsuperscript{137–139}. This includes culturing brain tumor cells as neurospheres and the use of specific growth factors such as EGF and b-FGF to preserve stemness of the cells. Cultured cells can be valuable for preclinical drug testing, however, these cells lack the microenvironment in which tumors grow in patients. Furthermore, the specific infiltrative growth patterns and limitations of drug delivery observed in patients cannot be mimicked using cultured cells. Therefore, in vivo models are likely to be more relevant to the clinical situation since tumor characteristics such as infiltrative growth can be mimicked more closely in these models.

In vivo models
Transgenic and xenograft in vivo mouse models are used to study brain tumor biology in a more clinically relevant setting as opposed to using cultured cells\textsuperscript{140–142}. Transgenic mice have genetically defined aberrations that result in the formation of specific brain tumors in these mice. Several of these models exist for a variety of brain tumor types, including GBM\textsuperscript{141,142}, medulloblastoma\textsuperscript{33}, and DIPG\textsuperscript{143}, and transgenic mouse models even exist for the different molecular subtypes of medulloblastoma\textsuperscript{33}.

Xenograft models are obtained by heterotopic or orthotopic transplantation of human cells in mice. Established brain tumor cell lines are often used for the induction of brain tumors in mice but these cells usually fail to mimic the infiltrative growth pattern of brain tumors\textsuperscript{141,142}. Direct transplantation of cells isolated from biopsies or injection of low-passage primary cell cultures in mouse brains does seem to recapitulate the characteristics of the original tumor more closely, although these models show an altered tumor immunology and absence of the natural microenvironment\textsuperscript{144,145}. These models represent important tools to study brain tumor biology and tumor progression including resistance to treatment. Furthermore, these models are highly valuable for preclinical drug testing especially when a panel of brain tumors is used to examine the heterogeneous treatment response of individual tumors.
IDENTIFYING NEW THERAPEUTIC INTERVENTION STRATEGIES

Our understanding of brain tumor biology is increasing, however, treatment outcome is still poor for patients with malignant brain tumors, especially for GBM and DIPG patients. Treatment resistance is a major problem in the management of brain tumors. Moreover, patients commonly suffer from severe treatment-related side-effects. There is an urgent need for novel treatment strategies that improve the efficacy of currently used treatment modalities, thereby also overcoming treatment resistance, or that allow lowering the dose of these conventional therapies. Preclinical research plays an important role in the search for novel therapeutic targets and agents and there are several approaches to identify these targets/agents. In the current –omics era, genomic, transcriptomic, proteomic, and epigenomic data obtained from patient material, cancer cell lines, and in vivo models are often employed for the identification of novel treatment targets. These datasets give insight into the molecular aberrant processes occurring in tumor cells, which could be targeted. Another strategy for the identification of novel targets/agents are unbiased drug screens, in which a large amount of drugs is screened for their capacity to induce cytotoxicity by themselves or only in combination with conventional therapies. In this thesis, we employed both –omics data (mRNA and miRNA profiles) and small molecule drug screens to identify novel targets and agents to improve efficacy of the conventional treatment modalities, chemo- and radiotherapy.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to elucidate mechanisms underlying treatment resistance in high-grade brain tumors, to interfere with these resistance mechanisms, and to improve the efficacy of currently used treatment modalities in GBM, medulloblastoma, and DIPG at the preclinical level.

In chapter 2, we employ gene expression profiling to identify genes, which are differentially expressed between GBM TMZ-sensitive cells and their TMZ-resistant subclones, in order to identify factors involved in TMZ resistance. Subsequently, we interfere with the signaling cascade of the identified factor EFEMP1 in an attempt to improve TMZ treatment efficacy in vitro and in vivo.

In chapter 3, integrative network analysis of miRNA and mRNA profiles of the same GBM TMZ-sensitive cells and TMZ-resistant subclones used in chapter 2 is performed to identify TMZ resistance factors. We use the integrative network analysis tool mirConnX to obtain the miRNA/mRNA networks. Subsequently, we examine if interfering with the expression of the identified resistance factor PHF6 resensitizes the cells to TMZ.

The GBM TMZ-sensitive and -resistant cells are used in a drug screen in chapter 4 to identify drugs that are able to improve TMZ efficacy. The TMZ-sensitizing effect of
the selected drug, hydroxyurea, is validated in vitro and in vivo and the underlying mechanism of resensitization to TMZ is assessed.

Radiotherapy has been proven as an effective treatment modality in some high-grade brain tumors, including medulloblastoma. However, treatment still fails in approximately 30% of patients and radiotherapy is accompanied by severe treatment-related side effects. In DIPG and GBM, almost all tumors recur after radiotherapy. Therefore, improvement of current treatment is necessary. In chapter 5, we perform a drug screen on medulloblastoma cells in combination with radiation to identify drugs that improve the efficacy of radiation. The radiosensitizing effect of the identified drug, quercetin, is, subsequently, confirmed in multiple medulloblastoma cells and in a mouse model of medulloblastoma.

In chapter 6, we determine if the clinically relevant WEE1 kinase inhibitor MK-1775 can be used to enhance the radiation response of DIPG cells.

Finally, the work presented in this thesis will be discussed in chapter 7 and future directions for further research will be given. In chapter 8 (English) and chapter 9 (Dutch) summaries are provided.

REFERENCES

82. Deutsch, M. et al. Results of a prospective randomized trial comparing standard dose neuraxis irradiation (3,600 cGy/20) with reduced neuraxis irradiation (2,340 cGy/13) in patients with low-


