**Brainbow**: Individual neurons of the dentate gyrus, a layer of the hippocampus, project their dendrites to the outer layer, where they receive input from the cortex. Neurogenesis occurs inside the "V," where new neurons are born and then migrate outward toward the dentate gyrus.
Chapter 1

General introduction
INTRODUCTION

Central nervous system (CNS) tumors are among the most heterogeneous types of tumors. Currently, CNS tumors are classified according to the 2007 World Health Organization (WHO) classification which includes over 130 subtypes on basis of morphology and biological behavior. This classification system grades CNS tumors into relatively benign, so-called low-grade tumors (WHO grade I or II) and malignant: high-grade tumors (WHO grade III or IV). The incidence of malignant CNS tumors is 3.3 per 100.000 children (age 0-19 years), and 8.9 per 100.000 adults (age >20 years). Among these malignant CNS tumors, medulloblastoma (MB) and glioblastoma (GBM) are the most common, in children and adults respectively. In recent years our understanding of the (epi)genetic background of these tumors has increased. However, outcome in these tumors remains poor and survivors suffer from severe late effects. Since the research discussed in this thesis focuses on MB and GBM, more background information on these tumor types is provided below.

Medulloblastoma

First described in 1925 by Cushing and Bailey as Spongioblastoma cerebelli, MBs are highly invasive primitive neuroectodermal tumors of the cerebellum. These tumors predominantly present in childhood. MBs constitute 15-20% of all primary childhood brain tumors. In adults MB is rare, with an incidence of 0.5 per million. Treatment for MB consists of surgical resection, and a combination of craniospinal irradiation and adjuvant chemotherapy. MBs are histopathologically subclassified into classic MB, desmoplastic/nodular MB, MB with extensive nodularity and large cell anaplastic MB. Based on the histopathological subtype, together with the presence of disseminated disease (Chang stage), and the volume of residual tumor following surgical resection, patients are divided into standard- and high-risk groups. Patients with high-risk disease are treated with more intensive radio- and chemotherapy than standard-risk patients. The five-year event free survival (EFS) for standard risk patients is 81-91%, while, the five-year EFS for high-risk patients is only 50%, despite intensive multimodality therapy. The developing nervous system is especially vulnerable to radiation and chemotherapy-induced damage, leading to severe neurocognitive impairment in children who survive the tumor and its treatment.

For the past two decades numerous studies have focused on the biological background of MB. Extensive gene expression profiling in large cohorts of MB samples has resulted in the identification of four clinically and molecularly distinct tumor subgroups. The first subgroup has aberrant activation of the Sonic Hedgehog (SHH) pathway and originates from granule neuron precursor cells (GNPs) of the developing cerebellum. The SHH pathway was first implicated in MB formation after the identification of germline mutations in PTCH1 gene in patients with Gorlin’s syndrome, a rare congenital condition that...
is characterized by an increased incidence of MB\textsuperscript{18,19}. Subsequent studies have identified several genomic alterations in components of the SHH signaling pathway in this MB subgroup\textsuperscript{20–23}. SHH signaling is known to drive proliferation in the GNPs of the cerebellum\textsuperscript{16}. Aberrant activation of the SHH pathway in GNPs leads to the formation of MB in mice, which genetically and histologically resembles the human SHH subgroup\textsuperscript{24–26}. This subgroup has an intermediate outcome, with a five-year survival rate between 60% and 80\%\textsuperscript{15,27}. The desmoplastic histology is exclusively seen in this subgroup and has a favorable outcome. However, the other histological subtypes are also seen in the SHH subgroup\textsuperscript{28}.

The second subgroup has activating mutations in the WNT pathway and arises outside the cerebellum from cells of the dorsal brainstem during early hindbrain development\textsuperscript{29}. The WNT pathway gene \textit{APC} is mutated in patients with Turcot’s syndrome, another familial brain tumor syndrome characterized by an increased incidence of MB and GBM\textsuperscript{30}. Approximately 10\% of sporadic MBs harbor mutations in components of the WNT pathway (\textit{APC, AXIN1, AXIN2} or \textit{CTNNB1})\textsuperscript{31–33}. WNT subgroup tumors are often of the classical histology and show infrequent metastatic dissemination. With a five-year survival of more than 95\% this subgroup has the most favorable outcome\textsuperscript{15,34,35}. The cell of origin for this MB subgroup was recently identified by overexpressing β-catenin (\textit{CTNNB1}) in lower rhombic lip cells located in the embryonic dorsal brainstem of mice\textsuperscript{29}. \textit{CTNNB1} activation resulted in MB formation. When comparing these tumors to human MB they genetically and histologically resembled human WNT subgroup MB\textsuperscript{26,29}.

For the other MB subgroups the pathological processes that drive tumor formation remain elusive, although these tumors show photoreceptor/GABAergic (Group 3) and neuronal/glutamatergic features (Group 4)\textsuperscript{12,13}. Group 3 MBs represents the most malignant subgroup with a five-year survival rate less than 50\%\textsuperscript{13,27}. These tumors more often show markers associated with poor outcome, such as large cell anaplastic histology, metastatic disease, and MYC amplification\textsuperscript{15}. Group 4 MB constitute the largest subgroup with intermediate outcome and predominantly classical histology\textsuperscript{15}. However, this subgroup is the most poorly understood.

**Glioblastoma**

GBMs are among the most aggressive cancers, with a five-year survival rate of less than 7.2\% in adults, and an inherent resistance to both chemo- and radiotherapy\textsuperscript{36}. The incidence of GBM is 3.19 per 100,000 people. The incidence increases with age, with most GBM arising after age 50 years, to 14.6 per 100,000 in the 75-84 years of age group\textsuperscript{2}. However, GBM also occurs in children with an incidence of 0.14 per 100.00 children (age 0-19 years)\textsuperscript{2}. Histologically, GBM is characterized by pleomorphic astrocytic cells, with increased mitotic activity, nuclear atypia, and poor differentiation with endothelial proliferation and areas of necrosis with surrounding pseudopalisading tumor cells\textsuperscript{37,38}.

GBM can arise \textit{de novo}, i.e. ‘primary’ (approximately 95\% of cases), or from lower-grade diffuse astrocytoma (WHO grade II) and anaplastic astrocytoma (WHO grade III), and is
then termed ‘secondary’ GBM (approximately 5% of cases). Primary GBM occurs more often in older patients, and is characterized by specific genetic alterations: amplification of \textit{MDM2}, \textit{PTEN} mutations, amplification of \textit{EGFR} or its mutant allele EGFRvIII. Secondary GBMs typically show \textit{TP53} mutations and \textit{PDGFRA} amplification. Mutations of \textit{IDH1} are characteristic for secondary GBM and are associated with better outcome.

The current treatment strategy for GBM patients consists of maximal surgical resection, radiotherapy and chemotherapy. Due to the infiltrative character of these tumors a complete surgical resection is typically impossible. However, a gross total resection of more than 97% is correlated with increased survival. Adjuvant radiotherapy in combination with temozolomide represents the standard treatment. Despite this multimodality approach survival outcome for adults with GBM is at 1, 2, 3, 4, 5 years 43.4, 17.9, 10.4, 8.4, 7.2% respectively. Conditional probability of survival of an additional year given survival to 1, 2, 3, 4, 5 years is 41.4, 58, 80.7, 85.7, 81.5% respectively. In children survival rates are slightly better with a five-year survival rate of 10 to 20%.

Multiple studies have used gene expression profiling to address the differences between pediatric and adult GBM, between primary and secondary GBM, and to identify subgroups within GBM. In pediatric GBM, a predominant PDGFRA driven gene signature was identified when compared to adult GBM, correlating with higher incidence of \textit{PDGFRA} amplification in pediatric GBM. Moreover, exome sequencing studies have identified age-specific recurrent mutations in \textit{H3F3A}, a gene encoding for Histone H3.3, and in genes encoding for H3.3-associated proteins. The use of integrated genomic profiling of adult GBM has resulted in the identification of four distinct GBM subgroups: proneural, neural, classical and mesenchymal, based on genetic and epigenetic differences.

The cells of origin from which GBM arise have not yet been established. However, several neural cell types have been suggested as a putative origin. Genetically manipulated neural stem cells, but also the more differentiated progeny such as oligodendrocyte precursor cells, and even mature glial cells and mature neurons, give rise to GBM. With the identification of the different GBM subgroups, one might speculate that each subgroup could have a unique cellular origin.

\section*{Need for novel therapies}

Despite increase in our knowledge, outcome in MB and GBM remains poor and survivors suffer from severe long-term treatment-related side effects. In MB the introduction of craniospinal irradiation increased survival rates to around 60%. However, higher doses of irradiation results in significant late effects in especially the younger patients, causing severe cognitive
impairment, secondary hormonal failure, and increased risk for secondary radiation-induced tumors. By introducing adjuvant chemotherapy in standard-risk patients the dose of craniospinal irradiation could be reduced without affecting outcome. Importantly, reducing the dose of craniospinal irradiation has improved cognitive outcome. In high-risk patients, the introduction of chemotherapy has improved outcome.

In GBM the effects of radiotherapy and chemotherapy have been more limited. The introduction of radiotherapy in the treatment of GBM following surgery has enhanced median survival from three to 12 months. reported that concomitant treatment with radiation and temozolomide, followed by adjuvant temozolomide in adults with GBM, increased median overall survival from 12.1 to 14.6 months, compared with that of patients receiving radiation alone. Furthermore, a subgroup of patients who responded better to temozolomide treatment was identified. Patients who had silencing of O(6)-methylguanine-DNA methyltransferase (MGMT) through promoter methylation had a median overall survival of 21.7 months compared with 12.7 months in those patients with no MGMT promoter methylation. However, outcome in GBM remains extremely poor due to this tumor’s aggressive behavior and resistance to conventional therapies.

A limitation of current treatment strategies remains the lack of specificity, as they rely mainly on conventional cytotoxic therapies which do not discriminate between cancer cells and normal cells. Thus, there is an urgent need to develop innovative therapies that can improve survival and reduce toxicity by selective targeting. In general, strategies for improving outcome fall into two broad categories: 1) selectively targeting tumor-specific pathways, and 2) targeting pathways responsible for resistance to conventional therapies.

AIM AND OUTLINE OF THIS THESIS

In recent years our knowledge on the molecular background of high-grade brain tumors has increased significantly. Using this knowledge we can now more precisely identify novel targets for MB and GBM. In this thesis we aimed to identify novel treatment targets for high-grade brain tumors (MB and GBM). These preclinical targets should then merge into future clinical trials. To do so we focused on three different processes driving high-grade brain tumors: 1) developmental pathways, 2) angiogenesis, and 3) therapy resistance. Developmental pathways are thought to play an important role in tumorigenesis and may provide therapeutic targets. For example, MBs are believed to originate from aberrantly dividing precursor cells, present during cerebellar development. Therefore, in chapter 2 the expression pattern of histone H3 lysine 27 trimethylation (H3K27me3) and its regulators EZH2 and KDM6B, during cerebellum development and in MB is investigated. GBM, on the other hand, are highly vascularized tumors, whose growth and infiltrative behavior are, for an important part, driven by angiogenesis. In chapters 3 and 4, the role of H3K27me3, EZH2 and microRNA-101 in GBM angiogenesis is investigated. GBMs have inherent resistance
to both chemo- and radiotherapy. Using in silico kinase expression analyses, in chapters 5 and 6, we identify WEE1 as driver of therapy-resistance in GBM.

Outline of this thesis

Chapter 1: Provides a general introduction on high-grade brain tumors, with special focus on MB and GBM and urges the need for identifying novel treatment targets.

Chapter 2: Explores trimethylation of H3K27me3 and the expression of the methylase EZH2 and the demethylase KDM6B during human cerebellar development and in medulloblastoma.

Chapter 3: Describes the role of EZH2 and the microRNA miR-101 in GBM proliferation, migration and angiogenesis.

Chapter 4: Further investigates the role of EZH2 and miR-101 in GBM angiogenesis by focusing on the interaction between GBM-associated endothelial cells and GBM cells.

Chapter 5: Reviews the role of WEE1 in controlling resistance to conventional therapies in cancers.

Chapter 6: Describes the role of WEE1-induced radiotherapy and chemotherapy resistance in GBM.

Chapter 7: General discussion and suggestions for future research.

Chapter 8: Summary of this thesis in English and Dutch.
REFERENCES


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