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Molecular Hallmarks of Gliomas

Marta Pojo and Bruno Marques Costa
Life and Health Sciences Research Institute (ICVS),
School of Health Sciences, University of Minho,
ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães
Portugal

1. Introduction

Tumors of the central nervous system (CNS) encompass a wide variety of entities, which span from benign to highly malignant. The classification of these tumors is typically based on their histopathological features or their location within the CNS. Despite these apparently simple criteria, there are a great number of independent CNS tumor types as defined by the most recent World Health Organization (WHO) classification of CNS tumors (Louis et al., 2007), which is the standard for the definition of CNS tumors worldwide. The WHO listing of CNS tumors is impressively vast and has, in fact, been surrounded by some controversy concerning the nosology of some tumor entities (e.g., the nosologic place of highly anaplastic oligoastrocytic tumors, glioblastoma with oligodendroglioma components). The age-standardized incidence rate of all primary non-malignant and malignant CNS tumors in the US is 16.5 per 100,000 person–years (9.2 per 100,000 person–years for non–malignant tumors and 7.3 per 100,000 person–years for malignant tumors) (CBTRUS, 2010). This rate is higher in females (17.2 per 100,000 person–years) than males (15.8 per 100,000 person–years). Worldwide data is available only for malignant primary CNS tumors; in this setting, the incidence rates are higher in males (approximately 3.7 per 100,000 person-years) than females (2.6 per 100,000 person–years) (Ferlay et al., 2008). In Western Europe, the male and female incidence rates of malignant CNS tumors are 6.7 per 100,000 person-years and 4.5 per 100,000 person-years, respectively. Very similar figures are observed in Northern America (6.0 and 4.5 per 100,000 person-years for males and females, respectively). Interestingly, the incidence rates are higher in more developed countries than in less developed ones, but these differences may be a consequence of differences in diagnostic practices, completeness of reporting and access to adequate health care, rather than attributable to geographic and genetic variation.

CNS tumors are considered to be primary when the tumor originally initiates in the CNS, as opposed to the far more common brain metastases derived from malignant tumors located in other organs, which are considered secondary brain tumors. Primary brain and CNS tumors account for only approximately 2% of all primary tumors (Louis et al., 2007), but they rank first among tumor types for the average years of life lost (~20 years, compared, for example, with ~6 years for prostate cancer and ~12 years for lung cancer) (Burnet et al., 2005). These tumors are the most frequent solid malignancy in children, being the leading cause of cancer-related death in children under the age of 19 (Rickert & Paulus, 2001). The impact and nature of primary brain tumors in adults is somewhat different, but they still rank second as cause of cancer death in males aged 20 to 39 years, and fifth in females of

those same ages (Jemal et al., 2009). This chapter will not fully review all the types of brain and CNS malignancies, but rather focus more specifically and thoroughly in gliomas, the most common type of all primary brain tumors.

2. Classification and epidemiology of gliomas

Glioma is a broad category of tumors divided into histological subgroups based on the type of glial cell of origin or morphological similarities between tumor and normal glial cells: astrocytomas (derived from astrocytes or their precursors), oligodendrogliomas (derived from oligodendrocytes or their precursors), and oligoastrocytomas (mixed lineage) are the three major subgroups, while ependymomas (derived from ependyma or their precursors) are less common (Louis et al., 2007). They represent 36% of all primary brain and CNS tumors, and account for approximately 81% of those that are malignant, an occurrence most likely related to the fact that the number of glial cells in the CNS is an order of magnitude higher than that of neurons. In addition, glial cells retain the ability to proliferative throughout adulthood, while most neurons do not, a characteristic that may also contribute to the higher incidence of this tumor group.

The WHO classification of CNS tumors is the most commonly used classification system among the medical and research community. This system classifies gliomas according to the presumed cell of origin based on similarities of tumor cells and non-neoplastic glial cells, as determined by histological analysis. Additionally, gliomas can be graded I to IV based on their degree of differentiation and malignancy (Louis et al., 2007), which can help predict the likely behavior of the tumor. Glioma grading is typically based on tumor cells' characteristics like mitotic index, nuclear atypia, vascular proliferation and necrosis (Louis et al., 2007). Simply put, grade I tumors often present well differentiated cells, are biologically benign and can be cured; grade II gliomas are malignancies that may follow long clinical courses, but early diffuse infiltration of the surrounding brain renders them incurable by surgery; grade III tumors exhibit increased anaplasia and proliferation, being more rapidly fatal; grade IV tumors exhibit more advanced features of malignancy, such as vascular proliferation and necrosis, and are generally lethal within 12 months. In general, grades I and II tumors are considered low-grade, while grades III and IV are high-grade tumors. The cell of origin of gliomas is a matter surrounded by controversy: for a long time, gliomas were believed to arise from differentiated glial cells, but this paradigm has recently been called into question, as some evidences suggest that these tumors arise from stem cells or lineage-committed progenitor cells (Singh et al., 2004). For the purposes of this chapter, the three major histological types of glioma (astrocytomas, oligodendrogliomas, and oligoastrocytomas) are discussed with particular emphasis.

2.1 Astrocytomas

Astrocytic tumors, or astrocytomas, are the most common intracranial neoplasms, accounting for approximately 75% of all gliomas (CBTRUS, 2010). Despite sharing a same cell of origin, astrocytoma is a broad category of tumors with different characteristics that differ in their location within the CNS, age and gender distribution, morphological features, growth potential, extent of invasiveness, tendency for progression, and clinical course. These are distinctly classified into four major clinicopathological entities, mostly based on their degree of malignancy: pilocytic astrocytomas (WHO grade I), diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III) and glioblastoma multiforme

(GBM, WHO grade IV). A brief summary of the main characteristics of these tumor types is given below, with a special emphasizes on the most common and malignant type, GBM.

Pilocytic astrocytomas (WHO grade I) account for ~5.8% of all gliomas, with similar frequencies in both males and females. Sometimes these are also referred to as "juvenile pilocytic astrocytoma", as they occur predominantly in children and teens, being the most common CNS tumor type among people under the age of 19 (CBTRUS, 2010). **Diffuse astrocytomas** (WHO grade II), sometimes called low-grade astrocytomas, account for ~1.5% of all gliomas and occur most commonly in young adults between the ages of 20 to 40. A slight male predominance (male-to-female ratio of 1.18:1) has been reported (Louis et al., 2007). This diffusely infiltrative nature may in part contribute to the typical progression of these tumors to more malignant neoplasms like anaplastic astrocytoma and, ultimately, glioblastoma. Three histologic entities can be distinguished: fibrillary astrocytoma (the most common), gemistocytic astrocytoma, and protoplasmic astrocytoma (Louis et al., 2007). **Anaplastic astrocytomas** (WHO grade III) are high-grade gliomas which may arise from a low grade diffuse astrocytoma or may arise *de novo*, i.e., without clinical evidence of a less malignant precursor lesion. These tumors account for approximately 7.5% of all gliomas, affect males more frequently than females (reported male to female ratios range from 1.1:1 to 1.6:1) and show a peak of incidence in adults aged between 30 to 50 years (Louis et al., 2007). In most cases, these grade III malignancies will progress to glioblastoma, showing a mean time to progression of ~2 years (Louis et al., 2007). **Glioblastoma** (WHO grade IV), or glioblastoma multiforme (GBM), is the most frequent and most malignant brain tumor, accounting for approximately 51% of all primary gliomas (CBTRUS, 2010). In Europe and North America, the incidence rates are approximately 3 new cases per 100,000 people per year. The peak of incidence occurs in adults aged 45 to 70 years, but glioblastoma can develop at any age. Males are more frequently affected (male to female ratio 1.7:1), and the frequency of glioblastoma in Caucasians is two times higher than in blacks. From a clinical and biological perspective, two main subtypes of this malignancy can be distinguished: primary (or *de novo*) glioblastoma develop without the presence of any precursor neoplastic lesion and manifest after a short clinical history (usually less than 3 months); secondary glioblastoma develop from lower grade tumors (a diffuse astrocytoma or an anaplastic astrocytoma) (Ohgaki & Kleihues, 2007). Primary glioblastoma, which are by far the most common, affect primarily older patients, present a quicker tumor progression and a poorer prognosis, as compared to secondary GBM. Of note, primary and secondary glioblastomas are histologically indistinguishable, but a growing body of data has been suggesting that these two disease entities evolve through different molecular mechanisms (as further detailed later in this chapter). Two histological variants of glioblastoma include: i) giant cell glioblastoma (WHO grade IV) is a rare variant (less than 5% of glioblastomas) composed of atypic multinucleated giant tumor cells; ii) gliosarcoma (WHO grade IV) accounts for approximately 2-8% of all glioblastomas (Homma et al., 2006; Meis et al., 1991), and is characterized by a biphasic tissue pattern with alternating areas displaying glial and mesenchymal differentiation.

2.2 Oligodendrogliomas

Oligodendroglial tumors account for approximately 8.4% of all gliomas, affecting males slightly more frequent than females (male to female ratio 1.1:1) (CBTRUS, 2010). The age distribution of oligodendrogliomas shows a peak of incidence in adults aged 40 to 50 years, but they may also rarely affect children (only 2% of all brain tumors in patients younger

than 14 years). Based on their degree of malignancy and other histopathological features, two histological subtypes are distinguished: oligodendroglioma (WHO grade II) and anaplastic oligodendroglioma (WHO grade III). These two entities encompass a continuum of neoplasms which range from well-differentiated to highly malignant (Louis et al., 2007). **Oligodendroglioma (WHO grade II)** account for approximately 6% of all gliomas (CBTRUS, 2010). **Anaplastic oligodendroglioma** (WHO grade III) accounts for approximately 2.4% of all gliomas (CBTRUS, 2010). Similarly to high-grade astrocytomas, anaplastic oligodendroglioma may develop *de novo* or by progression from a pre-existing low grade oligodendroglioma (WHO grade II).

2.3 Oligoastrocytomas (mixed gliomas)

Oligoastrocytomas are diffusely infiltrating gliomas that present with a mixture of two morphologically distinct tumor cell types, resembling the neoplastic cells present in astrocytomas and oligodendrogliomas (Louis et al., 2007). As such, these tumors are also often called "mixed glioma". Precise and consistent epidemiological data on oligoastrocytoma are not yet available, but they have been reported to account for approximately 2-9% of all gliomas, affecting preferentially males (male to female ratio 1.3:1). Median ages range from 35 to 45 years (CBTRUS, 2010). Oligoastrocytomas, like astrocytomas and oligodendrogliomas, are divided into low grade (WHO grade II) and high-grade (WHO grade III) oligoastrocytoma, based on the most aggressive type of neoplastic cell present in the tumor.

3. Etiology and risk factors for glioma

Typical risk factors for a variety of human tumors, including genetic and environmental factors (such as tobacco smoking, diet, excessive alcohol intake, occupational exposure to carcinogens, exposure to UV radiation, and some other personal habits), have not been consistently correlated with risk for glioma (Fisher et al., 2007). Epidemiological data suggest that approximately one third of all human cancers are related to dietary factors and lack of proper physical activity in adulthood. Additionally, a significant number of these could be prevented by vaccination (e.g., viral-related tumors) and/or behavioral changes (e.g., tobacco-related tumors and skin cancer), as their risk factors are well known. In contrast, reliable recommendations to prevent gliomas cannot be endorsed presently, as the etiology of glioma remains largely obscure. A brief summary of the most relevant potential risk factors for glioma are briefly reviewed below.

3.1 Environmental risk factors

Exposure to high-dose therapeutic radiation is the only firmly established exogenous environmental cause of glioma, which can clinically manifest only several years after the exposure (Ohgaki & Kleihues, 2005). Recent data suggest that use of high-dose chemotherapy to treat other tumors may also contribute to gliomagenesis (Edick et al., 2005). Interestingly, the degree of risk from these exposures to radiotherapy and chemotherapy may be influenced by the individual's genetic background; for example, a functional germline polymorphism in the thiopurine methyltransferase gene was shown to affect the susceptibility of children treated with cranial radiotherapy and intensive antimetabolite therapy for acute lymphocytic leukemia to further develop brain tumors (Relling et al., 1999). However, similar studies are needed to confirm these observations and extend its conclusions to adults.

Throughout life, people are exposed to a variety of endogenous and exogenous chemicals (for example, reactive oxygen and nitrogen species, *N*-nitroso compounds, several industrially used chemicals, and polycyclic aromatic hydrocarbons) through cellular metabolism, diet, occupation, and personal habits. Some of these compounds have been shown to be biologically plausible neurocarcinogens in a great number of animal and other studies; however, inconsistent or null findings have been reported for these same factors in human studies (reviewed in Wrensch et al., 2005). These inconsistent data among several association studies may result from a variety of issues, including small study sample sizes, false-positive results (due to both small sample size and lack of accurate statistical hypothesis), imprecise exposure measures (from proxy reporting and exposure history recall issues), protective exposures or conditions unaccounted for (e.g., allergies), variation in metabolic and repair pathways in the brain, differential diffusion of chemicals across the blood–brain barrier, and disease heterogeneity.

A currently hot topic is the potential association between cellular phone usage and glioma risk. While one study reported increased risk for glioma associated with long term (≥10 years) use of cellular phones (Hardell et al., 2007), other larger studies have failed to replicate this (Lahkola et al., 2007; Inskip et al., 2001; Fisher et al., 2007).

It is plausible that the failure to find strong and consistent associations between environmental risk factors and gliomas might be simply a reflection of absence of true associations (and not due to research issues). Nevertheless, considering the small number of studies examining environmental risk factors for specific well-defined glioma subtypes, it is premature to irrefutably conclude that environmental risks do not exist for glioma.

3.2 Genetic syndromes, familial aggregation, and mutagen sensitivity

Studies of genetic syndromes, familial aggregation, linkage, and mutagen sensitivity have suggested that genetics has an effect on susceptibility to glioma. Particular inherited rare genetic mutations cause a small number of genetic syndromes that have been associated with increased risk of glial tumors. Although these familial tumor syndromes account for a small proportion of cases (Ichimura et al., 2004), the elucidation of their molecular basis has greatly contributed to the understanding of gliomas, and has provided a valuable starting point for identifying candidate genes and pathways involved in gliomagenesis.

In large, carefully-designed epidemiological studies, familial glioma risks have been reported to be approximately two-fold, a magnitude that is similar to the familial association reported for other cancers for which susceptibility genes have been identified (e.g., breast cancer) (Malmer et al., 1999; Hemminki et al., 2000; Wrensch et al., 1997). The underlying causes of the pattern of familial brain tumor occurrence have been attributed to a variety of causes: one study implicates environmental factors only (Grossman et al., 1999), while others attribute multifactorial causes, polygenic causes, and autosomal recessive inheritance (de Andrade et al., 2001; Malmer et al., 2001).

Two case-control studies have suggested that peripheral lymphocytes from glioma patients are more sensitive to gamma-radiation than lymphocytes from matched controls, suggesting that increased sensitivity to radiation is an independent risk factor for gliomas (Bondy et al., 1996; 2001). The authors also propose that inherited genetic variation in the capacity to repair radiation damage may partly influence mutagen sensitivity and, ultimately, glioma risk.

3.3 Genetic polymorphisms

Since high-dose therapeutic radiation and inherited rare mutations explain only a small proportion of gliomas, neuro-oncology research has focused on genetic polymorphisms that, together with environmental risk factors, might potentially affect susceptibility to gliomas. Some of the most frequently studied polymorphisms, in the context of glioma, are in genes involved in DNA repair, carcinogen metabolism, cell cycle regulation, and immunological responses.

Because DNA repair is a mechanism of utmost importance in preserving genomic integrity, genes involved in this pathway have been extensively studied in human tumors. Glioma and some histological subtypes have been associated with specific polymorphic variants of particular DNA repair genes, such as *ERCC1* (excision repair cross-complementing 1), *ERCC2* (excision repair cross-complementing 2), *GLTSCR1* (glioma tumor suppressor candidate region gene 1), *PRKDC* (protein kinase, DNA-activated, catalytic), and *MGMT* (O^6-methylguanine-DNA methyltransferase) (Wang et al., 2004; Wiencke et al., 2005; Wrensch et al., 2005; Yang et al., 2005). However, the clear reliability of these findings is still requiring further studies to assess consistency. The DNA repair pathway is extraordinarily complex, as illustrated by the more than 130 genes known to be involved in base excision repair, direct reversal of damage, mismatch repair, nucleotide excision repair, homologous recombination, nonhomologous end joining, sanitization of nucleotide pools, activity of DNA polymerases, editing and processing of nucleases, and postreplicative repair (Wood et al., 2001). Studying genetic variants in this panoply of DNA repair genes might contribute to a better understanding of gliomagenesis, progression, and response to therapy (mostly DNA targeting agents).

Some of the most intriguing and consistent findings of the past decade of neuro-oncology research are the statistically significant inverse associations between risk of gliomas in adults and histories of allergies and/or chickenpox, IgG antibodies to varicella-zoster virus, and levels of serum IgE (Wiemels et al., 2004; Wrensch et al., 2001; 2005). A later study used germline polymorphisms in genes associated with asthma and allergies (*IL-4RA*, *IL-13* and *ADAM33*) as biomarkers for the presence of these conditions, as genetic polymorphisms could not be influenced by the presence of glioma. Remarkably, specific genotypic variants, which have been shown to increase asthma risk, were equally associated with decreased risk for glioblastoma (J. Schwartzbaum et al., 2005), further supporting the findings from self-reported case-control studies.

We have recently investigated genetic variants potentially relevant for glioma in the EGF/EGFR pathway, one of the most important growth signaling networks in human tumors, whose significance in gliomas has been well established. Previous studies have reached inconsistent conclusions regarding the relevance of a polymorphism in the *EGF* gene (*EGF*+61 A/G) as a risk factor for glioblastoma (Bhowmick et al., 2004; Vauleon et al., 2007). Later, we showed that the G allele increases the risk for gliomas, including glioblastoma and oligodendroglioma (Costa et al., 2007). Importantly, we have conclusively showed that the G variant of *EGF*+61 is associated with increased promoter activity, which may partly explain its relevance for glioma risk. In the *EGFR* gene, three polymorphisms have been described as having a transcriptional regulatory function: two single nucleotide polymorphisms in the essential promoter region, -216G/T and -191C/A, and a polymorphic $(CA)_n$ microsatellite sequence in intron 1. Preliminary studies by our group seem to indicate that the -191CA genotype is associated with higher risk for glioma, particularly oligodendroglioma, and that shorter $(CA)_n$ repeat variants are significantly associated with

increased risk for glioma, particularly glioblastoma and oligodendroglioma (Costa et al., 2010c). In contrast to a study by Carpentier et al. (2006), our preliminary data does not seem to implicate the -216G/T polymorphism as a risk factor for glioblastoma.

Our understanding of cancer as a multi-factorial disease, in which several pathways contribute to various stages of tumorigenesis, warrants the need for more integrative studies to evaluate interactions among these pathways. Accordingly, the simultaneous study of DNA repair, detoxification metabolism, cell cycle, and immunological pathways will allow more proper evaluation of different molecular players in gliomagenesis.

4. Molecular pathology of glioma

During the past two decades, remarkable progress has been accomplished in the understanding of the molecular pathology of gliomas. Similarly to the multistep process of human tumorigenesis in other human tissues (Hanahan & Weinberg, 2000), malignant transformation of normal cells (either differentiated glial or stem or progenitor cells) into gliomas results from the sequential accumulation of molecular aberrations (Ohgaki & Kleihues, 2007; Furnari et al., 2007). Tumor-associated molecular dysregulation can occur at the genetic and epigenetic levels, including chromosomal gains or losses, gene mutation, amplification, deletion, DNA and histones methylation or demethylation, and transcriptional regulation (Ichimura et al., 2004); (Wen & Kesary, 2008). An overview of some important molecular pathways in the development of gliomas is summarized in **Figure 1**, and briefly discussed below.

4.1 Chromosomal aberrations

Cytogenetic studies of gliomas have identified several chromosomal regions with copy number alterations (deletions, amplifications, gains, and losses). Typically, the presence of loss of heterozygosity (LOH) and deletions in tumors might point to chromosomal regions with tumor suppressor genes (those that normally function to suppress tumor formation/progression), while amplifications and chromosomal gains might indicate regions with oncogenes (those whose function favors tumorigenesis). However, these hints may be more difficult to interpret; strikingly, some particular chromosomal regions are commonly gained or lost in glioma. To make things a little more complex, the definition of tumor suppressor genes and oncogenes is highly context-dependent (e.g., on the tumor's location and intrinsic characteristics), as some proteins play roles that, depending on the tissue and cellular context, may favor or hamper tumorigenesis (Haber & Harlow, 1997).

Combined loss of chromosome arms 1p and 19q is the most frequently detected aberration in oligodendroglial tumors (Jeuken et al., 2004). Grade II and grade III oligodendrogliomas are equally affected by these chromosomal lesions, suggesting an early role in oligodendroglial tumorigenesis. Since losses of chromosome 1p and 19q are intimately correlated (Jenkins et al., 2006), it is plausible that the corresponding putative tumor suppressor genes may be involved in biologically distinct pathways, which may act synergistically in oligodendroglial tumorigenesis. Allelic losses of chromosomes 9p and 10q, and homozygous deletion of *CDKN2A* on chromosome 9p21, are also frequent in anaplastic oligodendroglioma (WHO grade III) (Bigner et al., 1999; Ueki et al., 2002). Chromosomal aberrations are also present in the majority (~70%) of both primary and secondary glioblastoma, which present LOH of chromosome 10q (Behin et al., 2003).
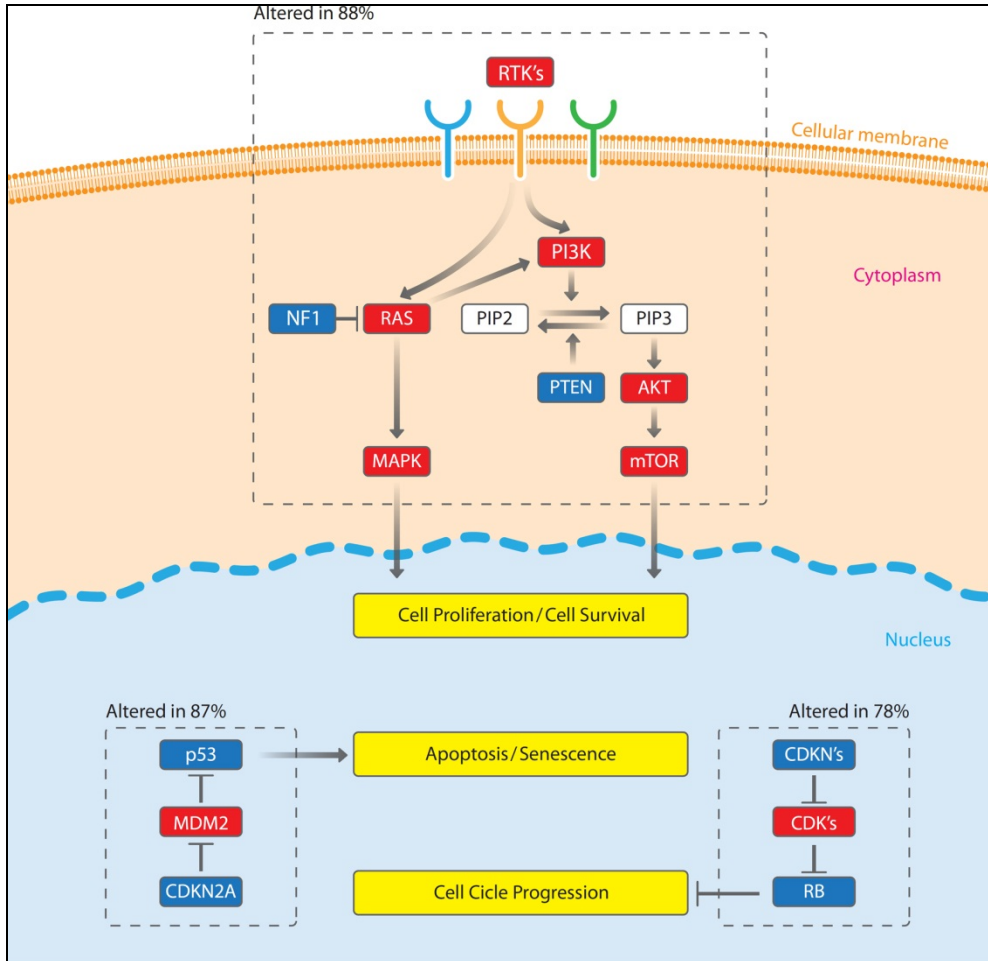
Fig. 1. Major signalling pathways commonly altered in glioblastomas: RTK/RAS/PI3K, p53, and RB. Red and blue boxes indicate proteins with activating and inactivating alterations, respectively, which can be genetic (gene amplifications or deletions, chromosomal copy number alterations and translocations, and mutations) and/or epigenetic (DNA CpG island hypermethylation, gene specific and global genome-wide DNA hypomethylation, and aberrant histone modifications). The frequency of alterations found in GBM in each pathway is indicated in the dashed boxes.

4.2 Dysregulated pathways

The histological heterogeneity of gliomas parallels with a substantial degree of molecular heterogeneity, both within and between histological subtypes of glioma (Rasheed et al., 1999; Freije et al., 2004). Several interconnected pathways are commonly altered in glioma, typically those governing cell-cycle regulation (cellular proliferation and senescence), cellular survival (apoptosis and necrosis), invasion (adhesion and migration), and

angiogenesis. These molecular abnormalities result in cell's self-sufficiency with respect to growth signals, insensitivity to growth-inhibitory stimuli, evasion of programmed cell death, "limitless" replicative potential, sustained angiogenesis, tissue invasion, and metastasis.

4.2.1 Receptor tyrosine kinase (RTK) pathways

Gliomas typically present overactivation of many mitogens (growth factors) and their specific membrane receptors. In order to proliferate, normal cells require activation of mitogenic signaling pathways (upon binding of diffusible growth factors to their specific transmembrane receptors) and subsequent intracellular signal transduction, which occurs mostly through the PI3K and MAPK signaling pathways. In contrast, acquired molecular alterations in tumor cells decrease their dependence on exogenous growth stimuli, facilitating their aberrant cell division, survival, and motility. While multiple mechanisms contribute to the ability of gliomas to overcome normal impositions on the control of mitogenic signaling (MAPK and PI3K/PTEN/AKT pathways, among others), abnormal activation of receptor tyrosine kinases (RTKs) seems to be the predominant (and better studied) mechanism.

Activation of receptor tyrosine kinase pathways in gliomas occurs through a variety of mechanisms, including overexpression of both ligands and receptors (leading to autocrine activating loops), gene amplification, and/or mutation of the receptor (resulting in constitutive activation even in the absence of the ligand). In addition, particular genetic polymorphisms may influence the expression of some of the genes involved in these pathways. The two most important receptor-driven signaling pathways in both CNS development and gliomagenesis are the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) pathways.

It is well established the importance of **EGF receptor (EGFR)** in development and differentiation of normal astrocytes (Burrows et al., 1997), but this RTK also plays critical roles in glial tumors (Kapoor & O'Rourke, 2003; Rao, et al., 2003). *EGFR* gene amplification is found in a variety of solid tumors, being notably frequent in astrocytomas (up to ~40% of all glioblastomas) (Viana-Pereira et al., 2008; Wong et al., 1992). These amplified versions of *EGFR* often undergo genetic rearrangements (e.g., *EGFRvI*, *EGFRvII*), some of which are relevant for the oncogenic and malignant roles of EGFR (Ekstrand et al., 1992). *EGFRvIII* (also known as Δ*EGFR*, or *EGFR**), which shows an in-frame deletion of exons 2–7, is the most common *EGFR* mutant allele, occurring up to 30% of all human GBM (and in up to 60% of those that have amplified wild-type EGFR) (Frederick et al., 2000). EGFRvIII protein, which exhibit constitutive ligand-independent tyrosine kinase activity, has been found to enhance tumorigenic behavior of human glioblastoma cells by reducing apoptosis and increasing proliferation (Nagane et al., 1996) and to malignantly transform murine *Ink4a/Arf* null neural stem cells (NSCs) or astrocytes in the mouse brain (Holland et al., 1998; Bachoo et al., 2002) making it an attractive and validated therapeutic target in glioma.

PDGF receptor-α (PDGFR-α), along with its specific ligands PDGF-A and PDGF-B, are also commonly overexpressed in gliomas, particularly in high-grade tumors (Westermark et al., 1995). In contrast, strong expression of PDGFR-β occurs mostly in proliferating endothelial cells in glioblastoma. PDGF-C and PDGF-D are also frequently expressed in glioma cell lines and in glioblastoma tissues (Lokker et al., 2002). Autocrine and paracrine signaling loops may be the primary means by which the PDGFR axis exerts its effects in gliomas, as these tumors often demonstrate coexpression of both PDGF ligands and receptors. The

importance of this signaling axis in gliomas has been supported by several recent studies: PDGF was shown to stimulate NSCs in the adult subventricular zone that express PDGFR-α to form glioma-like lesions in the mouse (Jackson et al., 2006); in addition, mice transgenic for neural progenitor PDGF-B expression resulted in the formation of oligodendrogliomas and forced elevation of PDGF-B levels increased overall tumor incidence (Dai et al., 2001; Shih et al., 2004). This growing body of data suggests that targeted therapy against this pathway could have therapeutic potential.

4.2.2 The PI3K-AKT pathway

Along with alterations in cell cycle regulatory pathways and RTK pathways, **intracellular signaling pathways** are also critical in glioma. Some important examples include the MAPK pathway (which plays a wide variety of cellular functions, such as regulation of cell-cell interactions, composition of extracellular matrix, and expression of genes promoting cell cycle progression (Dhanasekaran & Johnson, 2007), and the PI3K/AKT pathway (involved in an extraordinary diverse group of cellular functions, including cell growth, proliferation, differentiation, motility, survival and intracellular trafficking). Gliomas typically show increased activity of several molecular components of the MAPK and PI3K/AKT pathways, while some tumor suppressor genes that negatively regulate these pathways are often inactivated (Konopka & Bonni, 2003). For example, inactivating mutations in the tumor suppressor gene phosphatase and tensin homolog deleted on chromosome 10 (PTEN), whose protein is a negative regulator of the PI3K/AKT pathway, contribute to the abnormal activity of the pathway.

The **class IA PI3Ks** are heterodimers consisting of one regulatory subunit [one of five isoforms encoded by three genes: p85α, p55α, and p50α (*PIK3R1*); p85β (*PIK3R2*); and p55γ (*PIK3R3*)] and a catalytic isoform [p110α (*PIK3CA*), p110β (*PIK3CB*) and p110δ (*PIK3CD*)]. Activated RTKs recruit class IA PI3Ks to the membrane through their regulatory subunit. Activation of class IA PI3Ks at the membrane results in phosphorylation and conversion of the lipid phosphatidylinositol-(4,5)-bisphosphate (PIP_2) into phosphatidylinositol-(3,4,5)-triphosphate (PIP_3), which in turn recruits and activates phosphatidylinositol-dependent kinase 1 (PDK1). Activated PDK1, in turn, phosphorylates and activates protein kinase B (PKB, also known as AKT). A subsequent signaling cascade downstream of AKT ultimately results in inhibition of cell cycle arrest and apoptosis, and increased cell proliferation and survival. The tumor-suppressor phosphatase and tensin homologue (PTEN) negatively regulates PI3K signalling by dephosphorylating PIP_3, converting it back to PIP_2 (Hawkins et al., 2006).

The class IA PI3Ks are currently defined by their catalytic isoform, as the regulatory subunits appear to be functionally equivalent. Several studies have implicated class IA *PI3K*s genes in glial tumors. Gain-of-function mutations in the *PI3KCA* gene have been found in some malignant gliomas (e.g., glioblastoma), with a reported mutation frequency up to 15% (Samuels et al., 2004; Gallia et al., 2006). Inactivation of **PTEN** by mutations or epigenetic mechanisms is a frequent event in high-grade gliomas (up to 50%), which results in uncontrolled PI3K signaling **(**Knobbe & Reifenberger, 2003; Ohgaki et al., 2004). Studies using elegant mouse models have recently shown that the specific inactivation of PTEN in the mouse brain caused its overgrowth and abnormal proliferation of astrocytes (Fraser et al., 2004). Also in mouse studies, inactivation of PTEN has been associated with increased angiogenesis, closely paralleling the progression from low-grade to high-grade astrocytomas in humans, which coincides with *PTEN* loss (Andrew Xiao et al., 2002; 2005).

Aberrant PI3K signaling commonly results in activation of **Akt** via phosphorylation of two key residues, T308 and S473, by PDK1 and the mammalian target of rapamycin (mTOR), respectively (Mora et al., 2004; Sarbassov et al., 2005). In fact, assessment of the phosphorylation status of these residues in Akt is often the method of choice for determining activation of the PI3K pathway in cell lines and primary tumors, including glioblastoma samples, 85% of which have been shown to exhibit activated Akt (Wang et al., 2004). Additional mechanisms by which Akt activation may become dysregulated include decreased expression of PHLPP (PH domain leucine-rich repeat protein phosphatase, which dephosphorylates S473 in Akt), decreased expression of CTMP (C-terminal modulator protein, which binds to Akt and inhibits its phosphorylation), or overexpression of PIKE-A (phosphatidylinositol-3-kinase enhancer, which binds directly to phosphorylated Akt and enhances its anti-apoptotic function) in primary glioblastoma and glioma cell lines (Ahn et al., 2004).

Despite most of the signaling pathways previously mentioned (p53, RB, RTK, MAPK, and PI3K/AKT pathways) are often considered as distinct entities, there is significant cross-talk and inter-dependence among them, which reinforces the inappropriate regulation of any single pathway perturbation. (Indeed, these pathways are sometimes referred to as genetic networks, to emphasize their highly complex relationships.) For example, since p53 enhances transcription of *PTEN* (Stambolic et al., 2001), the loss of p53 in cells with constitutively active RTK signaling can further potentiate PI3K/AKT pathway activation.

The *NF1* tumor suppressor gene encodes neurofibromin-1, which functions primarily as a RAS negative regulator, and also plays a role in adenylyl cyclase and AKT-mTOR pathways. Loss of *NF1* is a typical molecular aberration of WHO grade I pilocytic astrocytoma (Zhu & Parada, 2002), and is believed to be one of the precursor lesions in these tumors, as the absence of negative regulation of RAS oncoprotein may favor cell proliferation and, ultimately, tumor initiation.

4.2.3 Cell cycle regulators

Genes involved in **cell cycle regulation** have been frequently found mutated in glioma, highlighting the importance of such genes in cellular proliferation and senescence. The RB and p53 pathways, which regulate the cell cycle primarily by governing the G1-to-S-phase transition, are major targets of functional inactivation in glioma, through any of several molecular alterations. The inactivation of these key regulators renders tumors highly sensitive to uncontrolled cell proliferation driven by mitogenic signaling effectors, such as phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK).

The RB protein exerts its anti-proliferative effects in quiescent cells by binding and sequestering the E2F family of transcription factors, preventing the transactivation of genes essential for cell cycle progression (Sherr & McCormick, 2002). Gliomas circumvent the RB-mediated negative regulation of the cell cycle by any of the following genetic/epigenetic aberrations: mutation of the *Rb1* gene, loss of its chromosomal region (13q) when progression from low- to high-grade gliomas occurs, amplification of cyclin-dependent kinases 4 and 6 (*CDK4* and *CDK6*) genes (which encode negative regulators of RB activity), and inactivation of p16^{Ink4a} (a negative regulator of CDKs) by allelic loss or DNA hypermethylation in high-grade gliomas (Louis et al., 2007). Even though the neutralization of the RB pathway alone is not sufficient to abrogate cell cycle control to the extent needed for cellular transformation (suggesting that other important cell cycle regulation pathways complement its activities in preventing gliomagenesis) (Xiao et al.

2002), the importance of its inhibition in progression from low- to higher-grade gliomas is underscored by the near-universal and mutually exclusive alterations of the molecular players of the RB pathway in both primary and secondary glioblastoma (Schmidt et al., 1994; Ueki et al., 1996).

The p53 tumor suppressor prevents the proliferation of cells with faulty genomes, mostly by halting the cell cycle in the G1 phase or by induction of an apoptotic program (Vousden & Lu, 2002). In sporadic (non-hereditary) gliomas, the p53 pathway is almost universally altered. A variety of molecular mechanisms may result in defective p53 function in glioma. Loss of p53 through either inactivating mutations in its DNA-binding domain or loss of chromosome 17p are common early events in the progression of secondary glioblastoma (Louis, 1994); in addition, germline p53 mutations in Li-Fraumeni patients, who have increased risk to develop gliomas, also underscore the importance of p53 in gliomagenesis (Malkin et al., 1990; Srivastava et al., 1990). Alternative inactivating alterations of the p53 pathway in gliomas include deletion of *p14^{ARF}* (whose protein stabilizes p53), amplification of the chromosomal region containing *MDM2* and *MDM4* genes (which encode negative regulators of p53 activity and expression) (Riemenschneider et al., 1999), and hemizygous deletion of the tumor suppressor gene *CDH5* (whose protein plays a role in maintaining *TP53* expression) (Bagchi et al., 2007).

4.2.4 Isocitrate dehydrogenases (*IDH1* and *IDH2*) genes

Very recent studies have identified mutations in the genes encoding the cytosolic and mitochondrial isoforms of NADP$^+$-dependent isocitrate dehydrogeneses (IDH1 and IDH2, respectively) in GBMs (Balss et al., 2008; Parsons et al., 2008; Ichimura et al., 2009; Watanabe et al., 2009; Yan et al., 2009; Zhao et al., 2009; Pollack et al., 2010; Christensen et al., 2011). These enzymes catalyze the decarboxylation of isocitrate to α-ketoglutarate (α-KG), in a reaction that utilizes NADP+ and produces NADPH. This cytosolic NAPDH is essential for the regeneration of reduced glutathione, which is important in the protection of cells against oxidative damage. These mutations in *IDH1* and *IDH2* are heterozygous and of somatic origin.   Until now, the mechanisms by which *IDH* mutant proteins contribute to gliomagenesis are not fully understood. The IDH1 R132H mutant variant, which is present in more than 90% of gliomas but rare in pilocytic astrocytomas, presents impaired substrate affinity, and dominantly inhibits wild-type IDH1 activity through the formation of catalytically inactive heterodimers (Zhao et al., 2009). The overexpression of mutant IDH1 was shown to reduce the formation of α-KG, and to increase the levels of HIF-1α, a critical transcription factor that promotes tumor growth under hypoxic conditions and whose stability depends on the levels of α-KG. This HIF-1α regulation by mutant IDH1 suggested that this mutation may cause oncogenic gains of function. Indeed, a later study by Dang et al., (2009) showed that mutant IDH1 converts a α-KG to 2-hydroxyglutarate (2-HG) in a NADPH-depend manner. Because mutations in *IDH1* and *IDH2* genes are restricted to a single amino acid, their detection for diagnostic purposes should be straightforward. Gliomas of astrocytic or oligodendrocytic lineage with *IDH* mutations have been shown to present distinct clinical and genetic characteristics as compared with gliomas with wild-type *IDH* genes (Yan et al., 2009), suggesting this mutation may be an early event in gliomagenesis from a stem/progenitor cell that can differentiate into both astrocytes and oligodendrocytes. Since *IDH* mutants occur in the large majority of WHO grade II or III gliomas, and in WHO grade IV secondary glioblastomas that develop from these lower-

grade precursor lesions, suggests that tumors with mutated IDH's encompass a specific subgroup of glioblastomas.

4.2.5 Molecular pathways to glioblastoma

As previously mentioned, glioblastomas can be subdivided into primary or secondary GBMs, based on their clinical and biological differences. Primary GBMs are by far the most common, affect older patients, and are genetically characterized by *EGFR* amplification and mutations, LOH of chromosome 10q, and deletion of the *PTEN* and the *p16^INK4a* genes (Louis et al., 2007). Secondary GBMs affect younger patients who had previously been affected by a lower grade astrocytoma, and are molecularly characterized by mutations in the *TP53* gene, overexpression of PDGFR, abnormalities in the p16 and Rb pathways, and LOH of chromosome 10q (Louis et al., 2007). Importantly, some of these molecular abnormalities are not exclusive for a specific glioblastoma subtype, but are rather present at significantly different frequencies (for example, the frequency of *TP53* mutation in secondary GBM is more than ~65%, but only ~28% in primary GBM) (Ohgaki & Kleihues, 2007). Despite being morphologically and clinically indistinguishable, as reflected by an equally poor prognosis when adjusted for patient age, primary and secondary GBMs have markedly distinct molecular features, at the levels of gene transcriptional patterns and DNA copy number aberrations (Furnari et al., 2007). Thus, these molecular distinctions strongly suggest that these GBM entities may respond differently to targeted molecular therapies, warranting the need to change the current standardize clinical management of these truly distinct diseases.

4.2.6 Epigenetic changes in glioma

In addition to genetic aberrations, gliomas also present a variety of epigenetic alterations, defined as mitotically heritable changes in gene expression that are not due to changes in the primary DNA sequence. The most well-studied epigenetic changes in gliomas occur primarily at two levels: DNA methylation at CpG dinucleotides, and post-translational modifications of histone tails. Nevertheless, other epigenetic mechanisms include noncoding RNAs (*e.g.*, microRNAs and small nucleolar RNAs), accumulation of histone variants, and chromatin remodeling, and may also be altered in brain tumors. Typical changes in DNA methylation levels observed in gliomas, as in other human tumors, include CpG island hypermethylation (associated with tumor suppressor gene silencing), gene-specific hypomethylation (resulting in aberrant oncogene activation), and genome-wide hypomethylation (potentially leading to chromosomal instability, loss of imprinting, and uncontrolled cellular proliferation). The histone modifications are quite more complex: firstly, because of the great variety of modifications that can occur in different histones (including, methylation, acetylation, phosphorylation, ubiquitylation, sumoylation, among others), and secondly, because a single histone tail residue can be mono-, di-, or tri-methylated on a single, specific lysine. In general, epigenetics is just another layer of gene expression regulation that, when altered in specific genes, may favor tumor formation and progression. While genetic and epigenetic changes in gliomas have been mostly studied independently, there is evidence that these mechanisms interact on specific genes, signaling pathways, and chromosomal domains. A summary of some of the epigenetic changes previously found in gliomas is presented in Table 1, and elegantly reviewed by Nagarajan & Costello, 2009.

Glioma type	Subtype (WHO grade)	Genetic Alterations	Epigenetic Alterations
Astrocytic tumors	Pilocytic astrocytoma (I)	*NF1* loss	CpG island hypermethylation in tumor suppressor genes (*MGMT, PCDH-gamma-A11, EMP3, THBS1, RASSF1A, CRBP1, TMS1, p16, SLC5A8, RB, PTEN, TP53, p14^ARF, TIMP3*) Genome-wide ("global") DNA hypomethylation Aberrant levels of histone post-translational modifications (*e.g.,* acetylation, methylation)
	Diffuse astrocytoma (II)	LOH 17p; *TP53* mutations; *PDGFRA* amplification	
	Anaplastic astrocytoma (III)	Gains of chromosome 7; Mutations in *TP53, RB1, CDK4, CDKN2A*; Deletions of 6p, 9p, 11p, 22q	
	Gliobastoma (IV)	Amplification of *EGFR, p14^ARF, CDK4, MDM2, MDM4*; Homozygous deletion of *CDKN2A, RB1*; Mutation of *TP53, RB1, PTEN, IDH1, IDH2*; Allelic loss of 19q, 13q	
Oligodendroglial tumors	Oligodendroglioma (II)	LOH 1p/19q; T*P53* mutations	
	Anaplastic oligodendroglioma (III)	LOH 1p/19q; 10q deletion; *CDKN2A* Amplification *EFGR* Mutation of *IDH1*	
Oligoastrocytic tumors	Oligoastrocytoma (II)	LOH 1p/19q; *TP53* mutations	
	Anaplastic oligoastrocytoma (III)	LOH 1p/10q; Mutations of *IDH1, TP53*	

Table 1. Common genetic and epigenetic hallmarks of gliomas

5. Molecular prognostic factors of malignant glioma

Recent studies started to identify potential biological and molecular characteristics in the tumors that may have prognostic value and help in making therapeutic decisions (Phillips et al., 2006), although such studies clearly require validation in prospectively followed and uniformly treated patients. Until now, work on the identification of prognostic markers in gliomas grants reasons for both optimism and caution with respect to improvements in the diagnosis and treatment of patients. In practice, the multitude of studies on the identification of such markers has led so far to development of only one molecular test with clinical relevance: 1p/19q testing in oligodendrogliomas. Much more effort is required for the identification of markers that truly and consistently distinguish glioma patients in ways

that can assist therapeutic decisions. Below we briefly discuss the current most promising molecular prognostic factors in these tumors, which are summarized in Table 2.

Molecular Prognostic Marker	Glioma Type
Chromosome 1p/19q co-deletion	Oligodendroglioma and oligoastrocytoma
MGMT promoter methylation	GBM and anaplastic astrocytoma
Loss of chromosome 10	GBM
Activation of the PI3K/AKT pathway	GBM
Activation of MAPK members	GBM
EGFR mutation (EGFRvIII)	GBM
PTEN expression (wild-type)	GBM
Molecular signatures (proneural, proliferative and mesenchymal)	GBM
High expression of angiogenic genes	Diffusely infiltrating gliomas of all histologic types
Stem-cell like gene expression signatures	GBM
CHI3L1 (YKL-40) expression	GBM
PTEN and DLL3 expression	GBM
EGFR expression	GBM
HOXA overexpression	GBM
IDH1-2 mutations	All gliomas, but not very frequent in primary GBM

Table 2. Selected Molecular Prognostic Markers for Gliomas.

5.1 Malignant astrocytomas

O^6-methylguanine-DNA methyltransferase (MGMT, also known as AGT) is an important DNA repair enzyme that contributes to glioblastoma resistance to temozolomide. The methyl group at the O^6 position of guanine can be removed by MGMT, which is consumed by this event (Spiro et al., 2001). In a similar study to the one from the EORTC–NCIC, Hegi et al., 2005, examined the DNA methylation at the promoter region of the *MGMT* gene in tumor samples from glioblastoma patients. This epigenetic event silences *MGMT*, which decreases tumor cells' ability to repair temozolomide-induced DNA damage, resulting in increased susceptibility of the tumor cells to temozolomide. Hegi reported that glioblastoma patients with a methylated *MGMT* promoter who were treated with temozolomide had a significantly longer median overall survival (21.7 months) and a higher 2-year survival rate (46%) than patients without *MGMT* promoter methylation who were similarly treated with temozolomide (median survival of only 12.7 months and a 2-year survival rate of 13.8%), suggesting that glioblastoma patients whose tumors do not have a methylated *MGMT* promoter do not benefit from temozolomide. Despite the striking and encouraging findings supporting *MGMT* as a glioma prognostic marker and a specific predictor of temozolomide-based chemotherapy, there is a significant body of controversial data surrounding the reproducibility of such findings (summarized in Costa et al., 2010a). Much of the controversy around the prognostic value of MGMT is due in part to studies including very heterogeneous groups of patients, with different glioma histologies, grades, and treatment types often grouped together in the same analysis. Additionally, different studies analyzed

MGMT at different levels, including methylation by methylation-specific PCR, mRNA expression by RT-PCR, and protein levels by immunohistochemistry and quantitative immunofluorescence. Due to the widely acknowledged need of replicating Hegi's findings, we investigated the potential of *MGMT* methylation as a prognostic marker of glioblastoma in a set of 90 prospectively followed patients uniformly treated with postoperative temozolomide-based chemoradiation. In line with some other reports, we found a trend for longer overall and progression-free survival in glioblastoma patients whose tumors had *MGMT* promoter methylation, but the differences failed to reach statistical significance in our data set (Costa et al., 2010a).

Recent evidences are revealing striking similarities between developmental and tumorigenic processes, suggesting that some of the molecular regulatory mechanisms necessary for normal development may be equally relevant in tumors. Homeobox (HB) genes encode transcription factors that play critical roles during normal development. These genes are broadly divided into two classes: class I includes clustered homeobox (*HOX*) genes, while class II includes divergent HB (non-*HOX*) genes that are dispersed throughout the genome (McGinnis & Krumlauf, 1992). The expression of some *HOX* genes has been shown to be altered in leukemias and several types of solid tumors. In recent years, a growing body of data has been implicating the aberrant expression of *HOXA* genes as an important mechanism in the pathophysiology of malignant astrocytomas. A study by (Abdel-Fattah et al., 2006) assessed the expression status of all *HOX* genes in primary astrocytomas and non-tumoral brain specimens, showing that a portion of these genes are aberrantly overexpressed in malignant astrocytomas. However, the relevance of these genes in glioma pathogenesis, malignancy, and prognosis was not addressed in that study. Later, Murat et al., 2008, suggested *HOX* genes may be part of a glioma stem cell signature with prognostic significance in patients treated with chemo-radiotherapy. We have recently shown that several *HOXA* genes are preferentially overexpressed in high-grade rather than in low-grade primary astrocytomas, and we have implicated the reactivation of *HOXA9* expression in GBM as a novel, independent, and negative prognostic factor in 2 independent sets of GBM patients (Costa et al., 2010b). Additionally, *HOXA9* reactivation was particularly frequent in a subgroup of GBM with aberrant chromosomal domains of transcriptional activation encompassing the *HOXA* cluster. Importantly, we demonstrated the PI3K-associated epigenetic mechanism by which this domain of activation is reversible: activation of the PI3K pathway in GBM cells decreased the levels of histone H3 lysine 27 trimethylation through inhibition of EZH2, a key histone methyltransferase. Finally, we provided functional data on the effects of *HOXA9* expression in GBM cells and immortalized astrocytes, which support its pro-proliferative and anti-apoptotic properties. Our findings revealed prognostic and therapeutic implications for oncogenic expression of the developmental transcription factor HOXA9 in malignant brain tumors, including the evaluation of PI3K inhibitors, which are being extensively tested in clinical trials. Finally, a recent study by (Gaspar et al., 2010) showed that high levels of *HOX* genes expression was a signature of resistance to temozolomide in pediatric GBM cell lines; in addition, pediatric high-grade glioma patients whose tumors expressed *HOXA* genes (particularly *HOXA9* and *HOXA10*) presented significantly shorter survival. Taken together, these recent studies point out to a very relevant role of *HOXA* genes in rendering high-grade gliomas even more malignant and resistant to therapy, resulting in particularly lethal tumors.

A genomic study by Parson and colleagues in 2008 showed that a subset of GBM patients had *IDH1* R132H mutations, and that this mutation occurred mostly in younger patients (mean age 33 years in *IDH1* mutant patients versus 53 years for patients with wild-type

IDH1). In addition, this mutation was particularly frequent in secondary GBMs (5 out of 6 secondary GBMs versus 7 out of 99 primary GBMs with IDH1 mutation), and was significantly associated with an increase in patients overall survival (Parsons et al., 2008). Yan et al. (2009) subsequently showed that *IDH1* mutation was commonly present in a variety of gliomas, including diffuse astrocytoma, anaplastic astrocytoma, and oligodendroglioma. In addition, the authors also identified a mutation affecting the analogous aminoacid residue (R172) of the *IDH2* gene, which was present in tumors without *IDH1* mutation. Importantly, the presence of either *IDH* mutation was associated with better survival of GBM and anaplastic astrocytoma patients. Christensen et al. (2011) also verified that *IDH1* mutation occurs more frequently than *IDH2* mutation in gliomas, and showed that the presence of *IDH1* or *IHD2* mutation was significantly associated with better survival of glioma patients, independently of patient age, sex, and grade-specific histology.

5.2 Malignant oligodendrogliomas and oligoastrocytomas

Tumors with 1p/19q codeletion are particularly sensitive to chemotherapy with PCV, with response rates as high as 100%, whereas patients whose tumors do not present 1p/19q codeletion show response rates of 23-31% (Ino et al., 2001; Cairncross et al., 1998). Indeed, oligodendroglioma is the first CNS neoplasm in which a molecular signature (1p and 19q codeletion) has been categorically associated with patient outcome within the context of large clinical trials (Felsberg et al., 2004). Even though the reasons for such associations are still unclear, the status of 1p and 19q, rather than classic histological analysis, is currently used as an eligibility criterion in studies involving patients with pure or mixed anaplastic oligodendrogliomas. This truly reflects a paradigm shift in the design of clinical trials for patients with these tumors, and has been regarded as a key example of the value of molecular signatures as prognostic markers of disease. Again, codeletion of 1p/19q was associated with improved survival in both studies. Although most studies of patients with pure or mixed oligodendroglioma were performed with PCV chemotherapy, temozolomide is arguably likely to have similar activity with less side effects (van den Bent, 2007; Yung et al., 1999); however, studies comparing the efficacy of these two chemotherapeutic regimens are still lacking.

6. Conclusion

Human gliomas are particularly dramatic diseases, not only because of their high lethality, but also because their etiological and prognostic factors are still not fully understood. Integrating multi-disciplinary teams to collaborate in the identification of new molecular and exogenous glioma risk factors is urgent. Possibly even more important is to rapidly improve the clinical management of glioma patients. While the current therapies used for malignant glioma and patients' outcome are not satisfactory, the recent insights on the biological and clinical features of glioma offer appealing opportunities for the development of more effective targeted therapy. The use of comprehensive genome-wide approaches to analyze genetic and epigenetic alterations, as it is the case of The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/about/mission.asp), may foster our understanding of the complex mechanisms underlying the various layers of glioma biology (formation, progression, resistance to therapy, etc) and aid in the identification of new avenues for drug development, particularly targeted therapies. Indeed, recent developments in second generation sequencing technologies and its more widespread application hold promise to

achieve these goals. Much more effort is required for the identification of molecular markers that truly and consistently distinguish glioma patients in ways that can assist therapeutic decisions. The integration of clinical and molecular data, now becoming available using recently developed tools such as gene microarrays, proteomics, and molecular imaging, will take us to an era where more targeted and effective treatments may be developed and implemented. We are cautiously optimistic that the development of a consensual molecular marker set predictive of therapy response in malignant gliomas, particularly glioblastoma, can arise in the future. Such marker set would substantially change the clinical management of these patients as it could aid the neuro-oncologist in individualizing therapy for each patient. Particularly, such marker set could allow the identification of the subset of patients who are likely to benefit from a specific therapy alone. Conversely, the ability to prospectively identify patients who are not be likely to respond to such therapy would also allow those patients to make an informed decision to participate in clinical trials with alternative novel therapies, avoiding adverse effects of specific treatments in the absence of anticipated clinical benefit. Additionally, a more in depth knowledge of the molecular determinants of treatment resistance could be the rational for designing the next generation of clinical trials for these highly malignant tumors. Finally, the identification of true molecular markers is also invaluable in the first steps of new drugs' development. Despite all the gaps in our understanding, a large amount of information is now available about the clinical and biological behavior of gliomas, and the molecular pathways that are relevant in their genesis and progression. In the quest toward individualization of glioblastoma treatment, the discovery of particular tumor molecular features, such as the status of *MGMT* methylation, *IDH1-2* mutations, and *HOXA* genes expression, may be the initial building blocks of a panel of molecular markers that truly have clinical implications. The challenge in the future will not only be to discover additional molecular markers of glioblastoma, but also to integrate all this new knowledge in an interdisciplinary manner to create an intelligible puzzle, which allows a more rational and efficient fight against this disease.

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8. References

Abdel-Fattah, R. et al., 2006. Differential expression of HOX genes in neoplastic and non-neoplastic human astrocytes. *The Journal of pathology*, 209(1), pp.15-24.

Ahn, J.-Y. et al., 2004. PIKE-A is amplified in human cancers and prevents apoptosis by up-regulating Akt. *Proceedings of the National Academy of Sciences of the United States of America*, 101(18), pp.6993-8.

Bachoo, R.M. et al., 2002. Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer cell*, 1(3), pp.269-77.

Bagchi, A. et al., 2007. CHD5 is a tumor suppressor at human 1p36. *Cell*, 128(3), pp.459-75.

Balss, J. et al., 2008. Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathologica*, 116(1432-0533 (Electronic)), pp.597-602.

Behin, A. et al., 2003. Primary brain tumours in adults. *Lancet*, 361(9354), pp.323-331.

Bhowmick, D.A. et al., 2004. A functional polymorphism in the EGF gene is found with increased frequency in glioblastoma multiforme patients and is associated with more aggressive disease. *Cancer Research*, 64(4), pp.1220-1223.

Bigner, S.H. et al., 1999. Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization.*The American Journal of Pathology*, 155(2), pp.375-386.

Bondy, M.L. et al., 2001. Gamma-radiation sensitivity and risk of glioma. *Journal of the National Cancer Institute*, 93(20), pp.1553-7.

Bondy, M.L. et al., 1996. Mutagen Sensitivity and Risk of Gliomas: A Case-Control Analysis. *Cancer Research*, 56(7), pp.1484-1486.

Burnet, N.G. et al., 2005. Years of life lost (YLL) from cancer is an important measure of population burden--and should be considered when allocating research funds. *British Journal of Cancer*, 92(2), pp.241-245.

Burrows, R.C. et al., 1997. Response diversity and the timing of progenitor cell maturation are regulated by developmental changes in EGFR expression in the cortex. *Neuron*, 19(2), pp.251-67.

Cairncross, J. G. et al., 1998. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *Journal of the National Cancer Institute*, 90(19), pp.1473-1479.

Carpentier, C. et al., 2006. Polymorphism in Sp1 recognition site of the EGF receptor gene promoter and risk of glioblastoma. *Neurology*, 67(1526-632X (Electronic)), pp.872-874.

CBTRUS, 2010. CBTRUS - 2009-2010 Eighteen States Statistical Report Tables.

Christensen, B.C. et al., 2011. DNA methylation, isocitrate dehydrogenase mutation, and survival in glioma. *Journal of the National Cancer Institute*, 103(2), pp.143-53.

Costa, B.M. et al., 2007. Association between Functional EGF+61 Polymorphism and Glioma Risk. *Clinical Cancer Research*, 13, pp.2621-2626.

Costa, B.M. et al., 2010a. Prognostic value of MGMT promoter methylation in glioblastoma patients treated with temozolomide-based chemoradiation: a Portuguese multicentre study. *Oncology Reports*, 23, pp.1655-1662.

Costa, B.M. et al., 2010b. Reversing HOXA9 oncogene activation by PI3K inhibition: epigenetic mechanism and prognostic significance in human glioblastoma. *Cancer Research*, 70, pp.453-462.

Costa, B.M. et al., 2010c. Associations between functional EGFR polymorphisms and glioma risk. In *European Journal of Cancer Supplements*. Oslo, Norway, p. 25.

Dai, C. et al., 2001. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes & Development*, 15(15), pp.1913-1925.

de Andrade, M. et al., 2001. Segregation analysis of cancer in families of glioma patients. *Genetic Epidemiology*, 20(2), pp.258-70.

Denning, G. et al., 2007. A short N-terminal sequence of PTEN controls cytoplasmic localization and is required for suppression of cell growth. *Oncogene*, 26(27), pp.3930-40.

Dhanasekaran, D.N. & Johnson, G.L., 2007. MAPKs: function, regulation, role in cancer and therapeutic targeting. *Oncogene*, 26(22), pp.3097-9.

Edick, M.J. et al., 2005. Lymphoid gene expression as a predictor of risk of secondary brain tumors. *Genes, Chromosomes & Cancer*, 42(2), pp.107-16.

Ekstrand, A.J. et al., 1992. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. *Proceedings of the National Academy of Sciences of the United States of America*, 89, pp.4309-4313.

Felsberg, J. et al., 2004. Oligodendroglial tumors: refinement of candidate regions on chromosome arm 1p and correlation of 1p/19q status with survival. *Brain Pathology*, 14, pp.121-130.

Ferlay, J. et al., 2008. *GLOBOCAN 2008* IARC, eds., Lyon: International Agency of Cancer. Available at: http://globocan.iarc.fr.

Fisher, J.L. et al., 2007. Epidemiology of brain tumors. *Neurologic clinics*, 25(4), pp.867-90, vii.

Fraser, M.M. et al., 2004. Pten loss causes hypertrophy and increased proliferation of astrocytes in vivo. *Cancer Research*, 64(21), pp.7773-9.

Frederick, L. et al., 2000. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Research*, 60(5), pp.1383-1387.

Freije, W.A. et al., 2004. Gene expression profiling of gliomas strongly predicts survival. *Cancer Research*, 64(18), pp.6503-10.

Furnari, F.B. et al., 2007. Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes & Development*, 21, pp.2683-2710.

Gallia, G.L. et al., 2006. PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. *Molecular Cancer Research*, 4, pp.709-714.

Gaspar, N. et al., 2010. MGMT-independent temozolomide resistance in pediatric glioblastoma cells associated with a PI3-kinase-mediated HOX/stem cell gene signature. *Cancer Research*, 70(22), pp.9243-52.

Grossman, S.A. et al., 1999. Central nervous system cancers in first-degree relatives and spouses. *Cancer Investigation*, 17(5), pp.299-308.

Haber, D. & Harlow, E., 1997. Tumour-suppressor genes: evolving definitions in the genomic age. *Nature Genetics*, 16(4), pp.320-2.

Hanahan, D. & Weinberg, R.A., 2000. The hallmarks of cancer. *Cell*, 100(1), pp.57-70.

Hardell, L. et al., 2007. Long-term use of cellular phones and brain tumours: increased risk associated with use for > or =10 years. *Occupational and Environmental Medicine*, 64(9), pp.626-32.

Hawkins, P.T. et al., 2006. Signalling through Class I PI3Ks in mammalian cells. *Biochemical Society Transactions*, 34(Pt 5), pp.647-62.

Hegi, M. E. et al., 2005. MGMT gene silencing and benefit from temozolomide in glioblastoma. *The New England Journal of Medicine*, 352, pp.997-1003.

Hemminki, K. et al., 2000. Cancers in the first-degree relatives of children with brain tumours. *British Journal of Cancer*, 83(3), pp.407-411.

Holland, E. C. et al., 1998. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. *Genes & Development*, 12(23), pp.3675-3685.

Homma, T. et al., 2006. Correlation among pathology, genotype, and patient outcomes in glioblastoma. *Journal of Neuropathology and Experimental Neurology*, 65(9), pp.846-54.

Ichimura, K. et al., 2004. Molecular pathogenesis of astrocytic tumours. *Journal of Neuro-Oncology*, 70(2), pp.137-160.

Ichimura, K. et al., 2009. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neuro-Oncology*, 11), pp.341-347.

Ino, Y. et al., 2001. Molecular subtypes of anaplastic oligodendroglioma: implications for patient management at diagnosis. *Clinical Cancer Research*, 7, pp.839-845.

Inskip, P.D. et al., 2001. Cellular-telephone use and brain tumors. *N Engl J Med*, 344(2), pp.79-86.

Jackson, E.L. et al., 2006. PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron*, 51(2), pp.187-99.

Jemal, A. et al., 2009. Cancer Statistics, 2009. *Ca-A Cancer Journal for Clinicians*, 59(4), pp.225-249.

Jenkins, R.B. et al., 2006. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer Research*, 66, pp.9852-9861.

Jeuken, J.W.M. et al., 2004. Molecular pathogenesis of oligodendroglial tumors. *Journal of Neuro-Oncology*, 70(2), pp.161-81.

Kapoor, G.S. & O'Rourke, D.M., 2003. Mitogenic signaling cascades in glial tumors. *Neurosurgery.*, 52(6), pp.1425-1434.

Knobbe, C.B. & Reifenberger, G., 2003. Genetic alterations and aberrant expression of genes related to the phosphatidyl-inositol-3'-kinase/protein kinase B (Akt) signal transduction pathway in glioblastomas. *Brain Pathology (Zurich, Switzerland)*, 13(4), pp.507-18.

Konopka, G. & Bonni, A., 2003. Signaling pathways regulating gliomagenesis. *Current Molecular Medicine*, 3(1), pp.73-84.

Lahkola, A. et al., 2007. Mobile phone use and risk of glioma in 5 North European countries. *International Journal of Cancer. Journal International du Cancer*, 120(8), pp.1769-75.

Lokker, N.A. et al., 2002. Platelet-derived growth factor (PDGF) autocrine signaling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. *Cancer Research*, 62(13), pp.3729-3735.

Louis, D. N., 1994. The p53 gene and protein in human brain tumors. *Journal of Neuropathology & Experimental Neurology*, 53, pp.11-21.

Louis, D. N. et al., 2007. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathologica*, 114(2), pp.97-109. Available at: ISI:000248046800001.

Malkin, D. et al., 1990. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, 250(4985), pp.1233-1238.

Malmer, B. et al., 1999. Familial aggregation of astrocytoma in northern Sweden: an epidemiological cohort study. *International Journal of Cancer*, 81(3), pp.366-370.

Malmer, B. et al., 2001. Genetic epidemiology of glioma. *British Journal of Cancer*, 84(3), pp.429-34.

McGinnis, W. & Krumlauf, R., 1992. Homeobox genes and axial patterning. *Cell*, 68(2), pp.283-302.

Meis, J.M. et al., 1991. Mixed glioblastoma multiforme and sarcoma. A clinicopathologic study of 26 radiation therapy oncology group cases. *Cancer*, 67, pp.2342-2349.

Mora, A. et al., 2004. PDK1, the master regulator of AGC kinase signal transduction. *Seminars in Cell & Developmental Biology*, 15(2), pp.161-70.

Murat, A. et al., 2008. Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. *Journal of clinical oncology : Official Journal of the American Society of Clinical Oncology*, 26(18), pp.3015-24.

Nagane, M. et al., 1996. A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. *Cancer Research*, 56, pp.5079-5086.

Nagarajan, R.P. & Costello, J.F., 2009. Epigenetic mechanisms in glioblastoma multiforme. *Seminars in Cancer Biology*, 19(3), pp.188-97.

Ohgaki, H. et al., 2004. Genetic pathways to glioblastoma: a population-based study. *Cancer Research*, 64(0008-5472 (Print)), pp.6892-6899.

Ohgaki, H. & Kleihues, P., 2005. Epidemiology and etiology of gliomas. *Acta Neuropathologica*, 109(1), pp.93-108.

Ohgaki, H. & Kleihues, P., 2007. Genetic pathways to primary and secondary glioblastoma. *The American Journal of Pathology*, 170, pp.1445-1453.

Parsons, D.W. et al., 2008. An integrated genomic analysis of human glioblastoma multiforme. *Science*, 321, pp.1807-1812.

Phillips, H.S. et al., 2006. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*, 9, pp.157-173.

Pollack, I.F. et al., 2010. IDH1 mutations are common in malignant gliomas arising in adolescents: a report from the Children's Oncology Group. *Child's Nervous System*, pp.1433-0350.

Rao, R.D. et al., 2003. Genetic and signaling pathway alterations in glioblastoma: relevance to novel targeted therapies. *Frontiers in Bioscience*, 8, p.e270-e280.

Rasheed, B.K. et al., 1999. Molecular pathogenesis of malignant gliomas. *Current Opinion in Oncology*, 11(3), pp.162-167.

Relling, M.V. et al., 1999. High incidence of secondary brain tumours after radiotherapy and antimetabolites. *The Lancet*, 354(9172), pp.34-39.

Rickert, C.H. & Paulus, W., 2001. Epidemiology of central nervous system tumors in childhood and adolescence based on the new WHO classification. *Child's Nervous System*, 17(9), pp.503-511.

Riemenschneider, M.J. et al., 1999. Amplification and overexpression of the MDM4 (MDMX) gene from 1q32 in a subset of malignant gliomas without TP53 mutation or MDM2 amplification. *Cancer Research*, 59(24), pp.6091-6.

Samuels, Y. et al., 2004. High frequency of mutations of the PIK3CA gene in human cancers. *Science (New York, N.Y.)*, 304(5670), p.554.

Sarbassov, D.D. et al., 2005. Growing roles for the mTOR pathway. *Current Opinion in Cell Biology*, 17(6), pp.596-603.

Schmidt, E.E. et al., 1994. CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. *Cancer Research*, 54(24), pp.6321-6324.

Schwartzbaum, J. et al., 2005. Polymorphisms associated with asthma are inversely related to glioblastoma multiforme. *Cancer Research*, 65(0008-5472 (Print)), pp.6459-6465.

Sherr, C.J. & McCormick, F., 2002. The RB and p53 pathways in cancer. *Cancer cell*, 2(2), pp.103-12.

Shih, A.H. et al., 2004. Dose-dependent effects of platelet-derived growth factor-B on glial tumorigenesis. *Cancer Research*, 64(14), pp.4783-9.

Singh, S.K. et al., 2004. Cancer stem cells in nervous system tumors. *Oncogene*, 23(43), pp.7267-7273.

Spiro, T.P. et al., 2001. Temozolomide: the effect of once- and twice-a-day dosing on tumor tissue levels of the DNA repair protein O(6)-alkylguanine-DNA-alkyltransferase. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 7(8), pp.2309-17.

Srivastava, S. et al., 1990. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature*, 348, pp.747-749.

Stambolic, V. et al., 2001. Regulation of PTEN transcription by p53. *Molecular Cell*, 8(2), pp.317-25.

Trotman, L.C. et al., 2007. Ubiquitination regulates PTEN nuclear import and tumor suppression. *Cell*, 128(1), pp.141-56.

Ueki, K. et al., 1996. CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. *Cancer Research*, 56(1), pp.150-153.

Ueki, K. et al., 2002. Correlation of histology and molecular genetic analysis of 1p, 19q, 10q, TP53, EGFR, CDK4, and CDKN2A in 91 astrocytic and oligodendroglial tumors. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 8(1), pp.196-201.

van den Bent, M.J., 2007. Anaplastic oligodendroglioma and oligoastrocytoma. *Neurologic Clinics*, 25(4), pp.1089-109, ix-x.

Vauleon, E. et al., 2007. The 61 A/G EGF polymorphism is functional but is neither a prognostic marker nor a risk factor for glioblastoma. *Cancer Genetics and Cytogenetics*, 172(1), pp.33-7.

Viana-Pereira, M. et al., 2008. Analysis of EGFR overexpression, EGFR gene amplification and the EGFRvIII mutation in Portuguese high-grade gliomas. *Antiacancer Research*, 28, pp.913-920.

Vousden, K.H. & Lu, X., 2002. Live or let die: the cell's response to p53. *Nature Reviews. Cancer*, 2(8), pp.594-604.

Wang, H. et al., 2004. Analysis of the activation status of Akt, NFkappaB, and Stat3 in human diffuse gliomas. *Lab.Invest.*, 84, pp.941-951.

Wang, L.E. et al., 2004. Polymorphisms of DNA repair genes and risk of glioma. *Cancer Research*, 64(16), pp.5560-5563.

Watanabe, T. et al., 2009. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am.J Pathol.*, 174, pp.1149-1153.

Wen, P. & Kesary, S., 2008. Malignant gliomas in adults. *The New England Journal of Medicine*, 359(17), p.1850; author reply 1850.

Westermark, B. et al., 1995. Platelet-derived growth factor in human glioma. *Glia*, 15(3), pp.257-263.

Wiemels, J.L. et al., 2004. Reduced immunoglobulin E and allergy among adults with glioma compared with controls. *Cancer Research*, 64(22), pp.8468-73.

Wiencke, J.K. et al., 2005. Molecular features of adult glioma associated with patient race/ethnicity, age, and a polymorphism in O6-methylguanine-DNA-methyltransferase. *Cancer Epidemiology, Biomarkers & Prevention*, 14(7), pp.1774-1783.

Wong, A.J. et al., 1992. Structural alterations of the epidermal growth factor receptor gene in human gliomas. *Proceedings of the National Academy of Sciences of the United States of America*, 89, pp.2965-2969.

Wood, R.D. et al., 2001. Human DNA repair genes. *Science (New York, N.Y.)*, 291(5507), pp.1284-9.

Wrensch, M. et al., 2005. The molecular epidemiology of gliomas in adults. *Neurosurgical Prevention Focus*, 19(5), p.E5.

Wrensch, M. et al., 2005. ERCC1 and ERCC2 polymorphisms and adult glioma. *Neuro-Oncology*, 7(4), pp.495-507.

Wrensch, M. et al., 2001. Prevalence of antibodies to four herpesviruses among adults with glioma and controls. *American Journal of Epidemiology*, 154(2), pp.161-5.

Wrensch, M. et al., 1997. Familial and Personal Medical History of Cancer and Nervous System Conditions among Adults with Glioma and Controls. *The American Journal of Epidemiology*, 145(7), pp.581-593.

Xiao, A. et al., 2002. Astrocyte inactivation of the pRb pathway predisposes mice to malignant astrocytoma development that is accelerated by PTEN mutation. *Cancer Cell*, 1(2), pp.157-68.

Xiao, A. et al., 2005. Somatic induction of Pten loss in a preclinical astrocytoma model reveals major roles in disease progression and avenues for target discovery and validation. *Cancer Research*, 65(12), pp.5172-80.

Yan, H. et al., 2009. IDH1 and IDH2 mutations in gliomas. *The New England Journal of Medicine*, 360, pp.765-773.

Yang, P. et al., 2005. Polymorphisms in GLTSCR1 and ERCC2 are associated with the development of oligodendrogliomas. *Cancer.*, 103(11), pp.2363-2372.

Yung, W.K. et al., 1999. Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group. *Journal of clinical oncology: Official Journal of the American Society of Clinical Oncology*, 17(9), pp.2762-71.

Zhao, S. et al., 2009. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. *Science*, 324, pp.261-265.

Zhu, Y. & Parada, L.F., 2002. The molecular and genetic basis of neurological tumours. *Nature Reviews Cancer*, 2(8), pp.616-626.