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Alterations in *TP53* gene – Implications in Tumorigenesis Process and Prognosis in Central Nervous System Cancer

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Additional information is available at the end of the chapter

1. Introduction

1.1. *TP53* Mutations and CNS tumors

Central nervous systems (CNS) malignancies, as others cancers, are formed by the uncontrolled cell growth that involves the sequential accumulation of alterations in genes controlling cell proliferation, lifespan, responses to stress, relationships with neighbors, and gene homeostasis. These genetic alterations can be achieved by intragenic mutations, chromosome alterations or epigenetics modifications, all playing important role in the activation or inactivation of key genes, such as oncogenes and tumor suppressor genes. Some of these mutations can be most frequently encountered in specific cancers or group of cancers and correlated with tumor biologic behavior and have implications on diagnosis, prognosis or treatment [1].

Biomarkers are important oncology tools in diagnostic, monitoring disease progression, helping in determining prognosis and predicting therapeutic response. Biomarkers vary from specific proteins and antigens to unique genetic, epigenetic or cytogenetic profiles, but common to all markers is that they provide specific information to a disease process. They function as supplementary and rarely supplanting, the histopathologic examination of tissues that is still the mainstay of traditional oncologic pathology [2, 3]. For this reason, we intend to compile the vast information about the important contribution of *TP53* gene as a biomarker in CNS cancer genesis, progression, stratification, prognosis, treatment and its importance to future targeted therapies.

CNS cancers are heterogeneous diseases, arbitrarily grouped by the systems that are affected. The "WHO (World Health Organization) Classification of Tumors of the Central Nervous System" discriminates more than one hundred different diseases derived from different cell types, affecting patients of different ages, with a vast biological behavior and clinical implications. It is not our intention to describe the features of each CNS tumor. Hence, authors will follow the WHO classification for CNS tumors [4].

TP53 tumor suppressor gene is the most frequently mutated gene in human tumors and one of the most studied on different kinds of cancer. It is a large and complex gene located on chromosome site 17p13.1 (Figure 1). It has 11 exons along approximately 20.000bp. This gene codifies a protein with 393 amino acids in which different domains are responsible for diverse functions as exhibited on Figure 1. Genetic variations in this gene contribute to human cancers in many different ways. Firstly, somatic mutations are frequent in most cancers [5]: it is estimated that mutations in this gene are present in half of the human cancers. The antiproliferative role of p53 protein in response to various stresses and during physiological processes such as senescence makes it a primary target for inactivation [6], mainly by a combination of single-base substitution and loss of alleles [7]. Secondly, inheritance of a mutated TP53 causes predisposition to early-onset cancers including breast carcinomas, sarcomas, brain tumors, and adrenal cortical carcinomas, defining the Li-Fraumeni (LFS) and Li-Fraumeni-like (LFL) syndromes [8, 9]. Thirdly, TP53 is highly polymorphic in coding and noncoding regions and some of these polymorphisms have been shown to increase cancer susceptibility and to modify cancer phenotypes in TP53 mutation carriers [10].

Commonly, advanced stage or aggressive behavior cancers have a higher frequency of TP53 mutations [11, 12]. Moreover, in cancers with low mutation rates, p53 is often inactivated by alternative mechanisms, like protein degradation. TP53 allelic deletion is also observed in many tumors, resulting in the reduction of expression of tetramers and decreased expression of genes inhibiting cell growth [13]. The cancer-associated somatic mutations in TP53 are primarily missense substitutions (72.28%) nonrandomly distributed along the molecule, [14]. Over 90% of p53 mutations occur in the central DNA-binding-domain (Figure 1) into exons 4 – 9. These single aminoacid changes affect the transcriptional activity of the gene to various degrees; sometimes missense mutants may even acquire new functions [15, 16]. The TP53 mutational pattern has proved to be a clinically relevant "molecular sensor" of genotoxic exposure to environmental carcinogens and endogenous mutagens [17].

Among single-base substitutions, about 25% are C:G>T:A substitutions at CpG sites. CpG dinucleotides mutate at a rate 10 times higher than other nucleotides, generating transitions [18]. About 3%–5% of cytosines in the human genome are methylated at position 5′ by a postreplicative mechanism that is restricted to CpG dinucleotides and is catalyzed by DNA methyltransferases. The 5′ methylcytosine (5mC) is less stable than cytosine and undergoes spontaneous deamination into thymine at a rate five times higher than the unmethylated base. This process is enhanced by oxygen and nitrogen radicals, leading to a higher load of CpG transitions in cancers arising from inflammatory precursors such as Barrett's mucosa or ulcerative colitis [19, 20]. Among the 22 CpG of the DNA-binding domain (DBD), three hotspot codons (175, 248, and 273) represent 60% of CpG mutations and another five residues (196, 213,
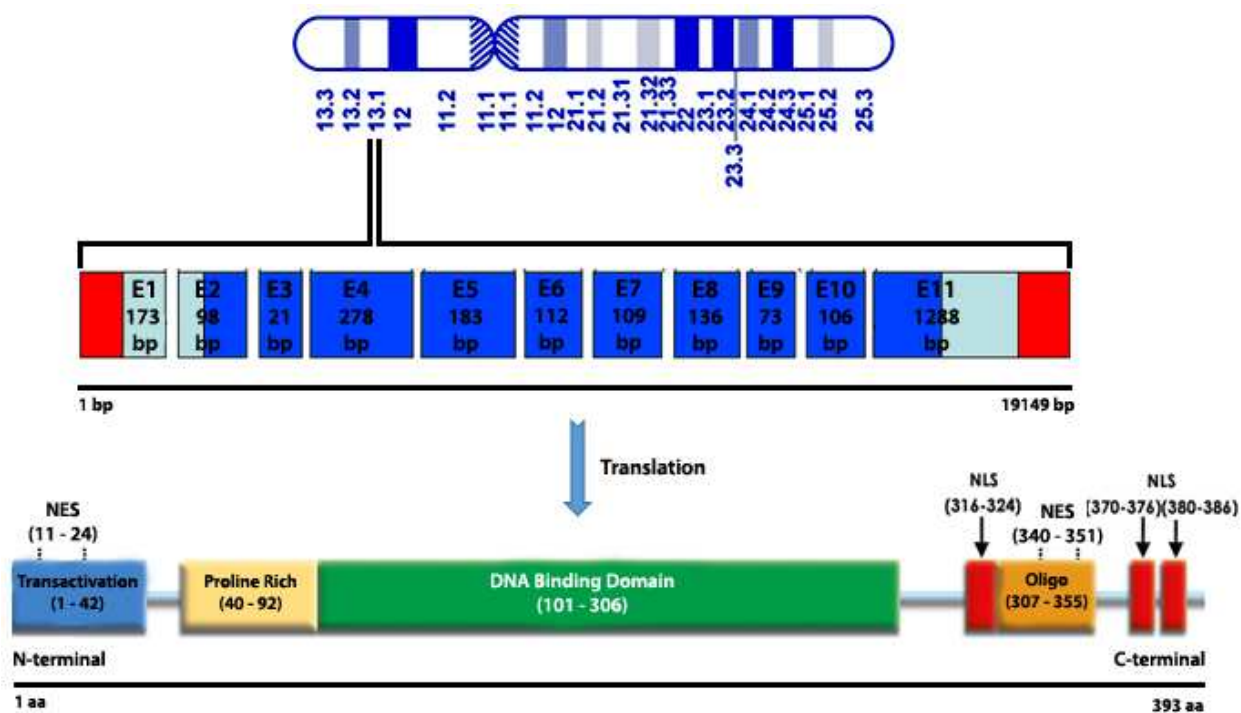
Figure 1. *TP53* gene: Structure, chromosome localization and protein domains distribution. *TP53* is mapped on human chromosome site 17p13.1. It is a long gene, with 19, 149 base pair comprising 11 exons that codify a protein with 393 amino acids long, in which the transactivation, proline-rich, DNA binding and the oligomerization domains are distributed. There are nuclear export/localization signals inside and between some domains (NES/NLS).

245, 282, and 306) account for 26% of these mutations. The lack of mutations at other CpG sites may reflect the fact that substitutions at these residues do not generate a dysfunctional protein. Although the same CpG hotspot mutations occur in many cancer types, other types of mutations tend to show differences among different cancers. Some of these differences have been linked to the effect of specific mutagens. This idea is endorsed by geographic differences which can be related to different environmental exposures [21].

All these mutational information about *TP53* are compiled in the International Agency for Research on Cancer (IARC) *TP53* Database [14], which provides structured data and analysis tools to study *TP53* mutations for specific cancers or investigate the functional and clinical impact of some mutations. The existence of this database, dedicated to annotate *TP53* mutations, polymorphism and respective implications in clinical and pathological behavior of human cancers, demonstrates the importance and the necessity of more knowledge to complete understand its implication on cancer [22].

Several studies have investigated the predictive value of *TP53* mutation status for tumor response to treatment and patient outcome in various cancers. However, different clinical and methodological settings have been used and the results have often been heterogeneous and contradictory [22]. The number and complexity of pathways in which *TP53* participates, the different mutational profiles of each cancer and the diverse environment conditions are variables that can contribute to these heterogeneous results.

The majority of mutations led to protein accumulation in the nucleus of the cells, which can be detected by immunohistochemistry (IHC) assays. Although some studies have shown an association between p53 positive immunostaining and poor outcomes, several studies have produced conflicting results and expectations on the use of p53 as a useful clinical biomarker failed [23]. Therefore, it seems IHC is a poor surrogate for gene mutation detection, as many mutations do not lead to protein accumulation, and because accumulation of wild-type p53 may also occur in the absence of gene mutation, producing a high rate of false negative and positive results. Hence, the use of IHC leads to an unacceptable number of misclassified cases and to a greater inter-study variability [1, 22].

By contrast, the screening for *TP53* mutations by gene sequencing, precisely identifying the mutation, have produced more consistent results, at least for some types of cancers such as breast, head and neck squamous cell carcinoma (HNSCC), and leukemia, in which the presence of a *TP53* mutation is associated with poor outcomes. In other types of cancer such as brain and pancreas, mutations were also found to be associated with both poor and good prognosis, depending on the study and cancer. These results show that the type of tissue and treatment may be important determinants of the prognostic and predictive value of *TP53* mutations [1, 22]. Figure 2 illustrates the use of different techniques in the evaluation of mutational status of *TP53* and expression of p53 protein in gliomas. Fluorescence *in Situ* Hybridization (FISH), sequencing and IHC techniques.

2. *TP53* genetic alterations in CNS tumors

CNS tumors have historically been classified on the basis of morphological and, more recently, immunohistochemical features with less emphasis on their underlying molecular pathogenesis. The past two decades, however, have seen striking advances in basic brain tumor biology, especially with regard to malignant gliomas and medulloblastomas, the most common CNS cancers of adults and children, respectively [24, 25]. Molecular signatures of tumors may play roles as diagnostic, prognostic, and predictive markers and influence the clinical decision making process. A dynamic classification of tumors is critical for the continuous integration of newly established molecular tools. This topic focuses on various genetics and epigenetics *TP53* changes in the CNS tumors which have been integrated into daily practice and gained significance for molecular diagnostic testing. Detailed discussion of neuronal and mixed neuronal-glial tumors, tumors of the pineal region, tumors of cranial and paraspinal nerves, mesenchymal tumors, lymphomas and haematopoietic neoplasms and other tumor entities is beyond the scope of this chapter, especially because there is only limited molecular information used in clinical management available for this types of tumors.

2.1. Gliomas

Gliomas are the most frequent primary brain tumors and include a variety of different histological types and malignancy grades. Although the cellular origin of gliomas is still unknown, experimental data in mice suggest an origin from neoplastically transformed neural
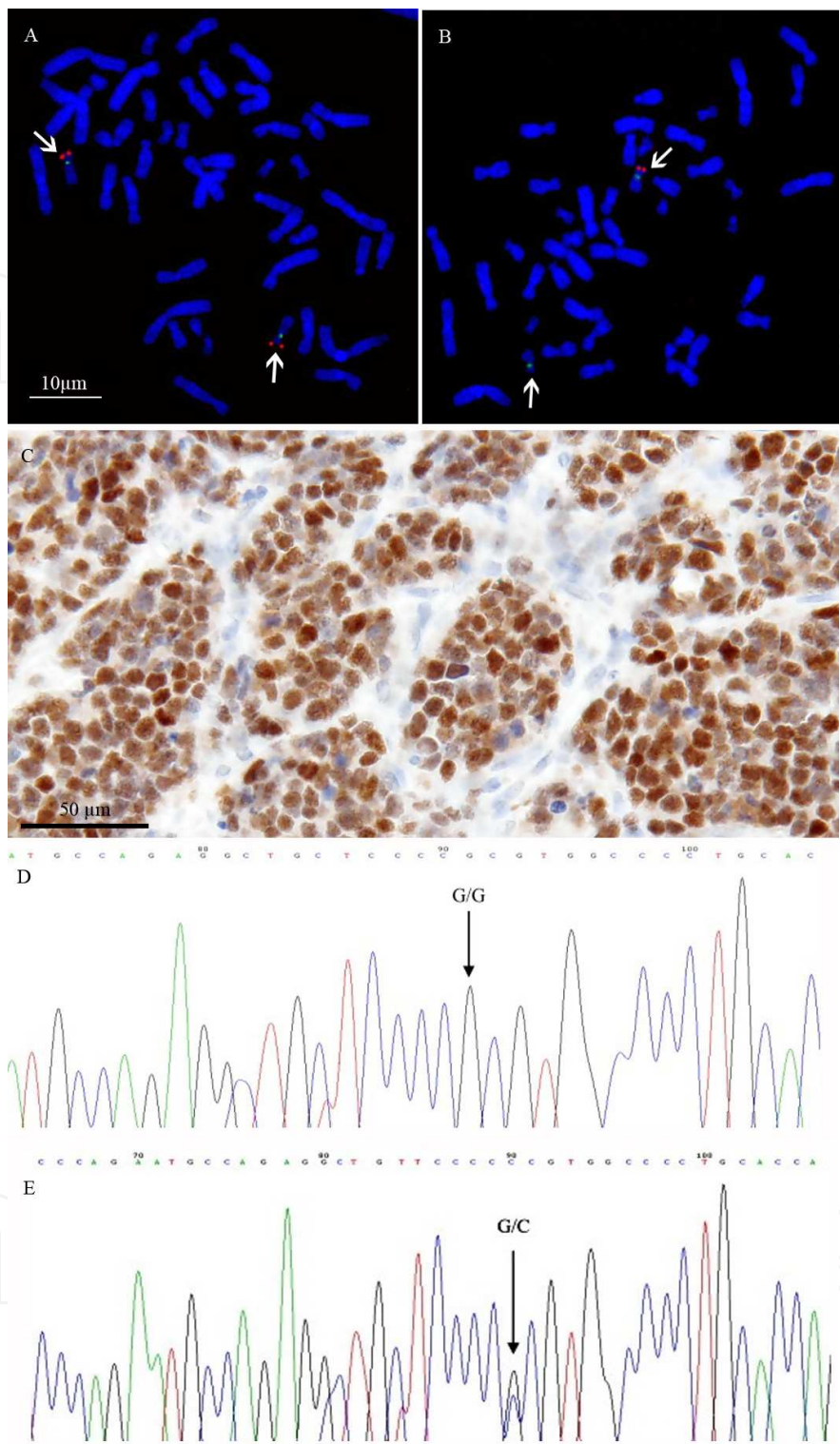
Figure 2. Different approaches used in the analysis of *TP53* gene in gliomas. (A) and (B) FISH experiments using *TP53* locus specific probe (red) and 17 centromeric probe (green) in metaphase chromosomes. A normal pair of chromosome 17 is showed in (A), while a heterozygous deletion of *TP53* can be observed in (B). (C) Immunopositive p53 sample, demonstrated by immunohistochemical staining. (D) Electropherogram of an patient with the wild type sequence (CGC) in the codon 72 while (E) illustrates a base exchange mutation in this position (CCC) predicting de aminoacid substitution arginine → proline.

stem or progenitor cells. However, histological classification of gliomas essentially relies on morphological similarities of the tumor cells with non-neoplastic glial cells and the presence of particular architectural features; thereby, most gliomas can be classified as astrocytic, oligodendroglial, mixed oligoastrocytic or ependymal tumors according to the criteria of the WHO classification of CNS tumors [4]. Clinical experiences derived from the prospective randomized clinical trials have shown that the histomorphological criteria alone might not be sufficient to predict the clinical outcome. Moreover, lately integrated genomic studies and exome sequencing have revealed the existence of multiple distinct molecular subtypes within histologically similar looking tumors [26]. For instance, even gliomas with identical histopathological features differ considerably regarding clinical course or response to therapy.

Knowledge of the genetic alterations in the various types and malignancy grades of gliomas has drastically increased over the past years. The evolution of classical tumor molecular and cytogenetic techniques, as well as the development of newer array-based assays of comparative genomic hybridization and RNA expression, allowed subclasses of gliomas to be identified based on molecular or gene expression patterns, showing substantial genetic and gene-expression heterogeneity within and between histologic grades of different histologic types of gliomas [27]. These approaches have identified point mutations and copy number changes (deletions, amplifications, gains) in several regions; deletions and loss of heterozygosity in tumors might point to genes involved in tumor suppression, whereas amplifications and gains might point to genes involved in initiation or progression processes (e.g. oncogenes) [28].

Numerous molecular abnormalities have been associated to the underlying biology of gliomas. The p53 pathway is nearly invariably altered in sporadic gliomas: loss of p53, through either point mutations that prevent DNA binding or deletion in chromosome 17p, is a frequent and early event in the pathological progression of secondary glioblastoma (GBM) [29, 30]. The importance of p53 in gliomagenesis is also underscored by the increased incidence of gliomas in LFS, a familial cancer-predisposition syndrome associated with germline p53 mutations [31]. This genetic linkage has been reinforced by a glioma-prone condition in mice engineered with a commonly observed Li-Fraumeni p53 mutation [32] as well as in p19^{ARF}-null mice, albeit at a low frequency [33]. In human gliomas, p53 mutations are primarily missense mutations and target the evolutionarily conserved domains in exons 5, 7, and 8, thus affecting residues that are crucial to DNA binding [30].

The finding that a second promoter drives an alternatively spliced transcript at the *CDKN2A* locus prompted the discovery of an additional tumor suppressor gene that is inactivated at this locus [34]. The second protein encoded by *CDKN2A*, p14 ^{ARF}, was subsequently shown to be an important accessory to p53 activation under conditions of oncogenic stress due to its neutralization of the p53 ubiquitin ligase, *MDM2* [35, 36], an oncogene originally discovered amplified as double minute chromosomes in a spontaneously transformed murine cell line, and then later found to be a key negative regulator of p53 during normal development and in tumorigenesis [37-39]. Concordantly, the chromosomal region containing *MDM2*, 12q14-15, is amplified in ~10% of primary GBM, the majority of which contain intact p53 [40]. The discovery of the *MDM2*-related gene, *MDM4* (chromosome 1q32), which inhibits p53 transcription and enhances the ubiquitin ligase activity of MDM2, prompted the finding that the

p53 pathway is also inactivated by the amplification of *MDM4* in 4% of GBM with neither *TP53* mutation nor *MDM2* amplification [41, 42]. Additionally, the recently discovered tumor suppressor gene *CHD5* (chromodomain helicase DNA-binding domain 5), which maps to chromosome 1p36 and is therefore frequently hemizygously deleted in those human gliomas with loss of 1p, has been shown to maintain p53 levels by facilitating expression of p19 ^{Arf} (mouse p14^{ARF} ortholog), and thus presents an additional mechanism for inactivation of this critical pathway [43].

2.1.1. Astrocytic tumors

The incidence of *TP53* mutations in pilocytic astrocytomas is controversial, with some authors reporting only infrequent mutations [44-47], while more common mutations are rare [48]. Hayes *et al.* [48], were the first to find a higher rate of *TP53* mutations in an analysis of 20 pilocytic astrocytomas in children, based on a comprehensive denaturing gradient gel electrophoresis mutation detection assay of the entire coding region, including all splice site junctions of *TP53*, showed mutations considered as causative in 7 of the 20 (35%) pilocytic astrocytomas. Few Cytogenetic studies have been carried out, showing allelic losses on both 17p and 17q including the *TP53* and *NF1* loci in pilocytic astrocytomas [49]. These results suggest that *TP53* mutations may well play a role in the development of these tumors.

TP53 mutations are a genetic hallmark of low-grade diffuse astrocytomas, for > 60% of these tumors carrying mutations in this gene [47, 50], mainly in gemistocytic astrocytomas with *TP53* mutations in up to 80% of the cases [51, 52]. In most cases, *TP53* mutation is accompanied by loss of heterozygosity (LOH) on 17p resulting in the complete absence of the wild-type *TP53* gene. Those diffuse astrocytomas with no *TP53* mutations may have altered the p53-dependent growth control by alternative mechanisms, for example, promotor methylation of the *p14* ^{ARF} gene at 9p21. Nakamura *et al*. [53] found hypermethylation of *p14^{ARF}* in one third of low-grade diffuse astrocytomas samples. These results suggest that aberrant *p14^{ARF}* expression due to homozygous deletion or promoter hypermethylation is associated with the evolution of both primary and secondary GBMs, and that *p14^{ARF}* promoter methylation is an early event in subset of astrocytomas that undergo malignant progression to secondary GBM.

Studies assessing the presence of *TP53* mutations as predictor of clinical outcome in diffuse astrocytomas have been made and the results are controversial. However most of them associated the presence of *TP53* mutations to a poor prognosis [51, 54, 55]. Peraud *et al.* [54] analyzed retrospectively timing, frequency, and prognostic impact of *TP53* mutations and p53 protein accumulation in 159 patients consecutively treated at a single neurosurgical clinic. *TP53* mutations were frequently found and univariate analysis found that gemistocytic subtype and *TP53* mutation were associated with worse prognosis, with only the gemistocytic subtype remaining an unfavourable prognostic factor on multivariate analysis. In non-gemistocytic astrocytomas, a mutation in *TP53* hot spot codon 175 indicated a worse prognosis in terms of time to progression and malignancy.

Xanthoastrocytoma pleomorphic (PXAs) are rare astrocytic malignancies classified as grade II lesions by the WHO. Because of the relative rarity of this lesion, the molecular background is still unclear. Among the abnormalities frequently observed in astrocytic tumors, PXA shares

only *TP53* mutations, and, although *TP53* mutations in anaplastic PXA have previously been reported, the significance of this alteration for tumor malignant progression is not clear [56, 57].The high frequency of *TP53* mutations in low-grade astrocytomas raises the question of whether these alterations play an important role in the tumorigenesis of PXA. Paulus and coworkers [58] reported the highest frequency of TP53 mutations, around 25%. However, in contrast, Giannini *et al.* [59] identified mutation in only 1 of 47 samples, all of which were nonrecurrent lesions and all lacked anaplastic transformation, while Bettegowda *et al.* [60] sequenced the exomes of 12 PXAs and identified mutation in only 2 cases.

Anaplastic astrocytomas, from a clinical, morphologic and genetic point of view, represents an intermediate stage on the route of progression to GBM. They exhibit high *TP53* mutation rate (40-70%) similar to diffuse astrocytomas and high frequency of LOH at 17p [61, 62]. In a study of almost 200 astrocytomas of grades II-IV, 72% of anaplastic astrocytomas were found to have a disruption in the p53 pathway [63].

An important clue to pathways involved in gliomagenesis may lie in the two GBM subtypes that have been clinically identified [50]. Primary GBM is typically present in older patients as aggressive, highly invasive tumor, usually without any evidence of prior clinical disease. Secondary GBM have a very different clinical history, being usually observed in younger patients who initially presented low-grade astrocytoma that transformed in GBM within 5–10 years of the initial diagnosis, regardless of prior therapy. The cataloging of genetic lesions in these GBM subtypes has identified differences in their genetic profiles, predominantly in the penetrance of specific genetic mutations. As a result, it has been proposed that primary and secondary GBMs represent two distinct clinical entities, each developing along distinct genetic pathways [50].

TP53 mutations are a genetic hallmark of secondary GBM, because these tumors have a high incidence of mutations in this gene (>65%), suggesting that p53 pathway plays a crucial role in their development tumors [62, 64-66]. *TP53* mutations are the first detectable genetic alteration in > 60% of precursor low-grade diffuse astrocytomas or in anaplastic astrocytomas in a similar frequency, and secondary GMBs derived thereof [64, 67]. *TP53* mutations also is present in primary GMBs, but with significantly lesser frequency (25-30% of cases) [47, 67]. Giant cell glioblastoma, a histological variant of GBM, carry *TP53* mutations in high frequency (75 – 90%) [68, 69], while gliosarcoma, another GBM variant characterized by a biphasic tissue pattern, has a lower *TP53* mutation rate (23–24%) [70, 71], and identical *TP53* mutations in both gliomatous and sarcomatous components [70].

In secondary GBMs, 57% of mutations have been reported to be located in the two hotspot codons 248 and 273; however, in primary GBMs, mutations were more equally distributed through all exons, with only 17% occurring in codons 248 and 273 [67]. Furthermore, G:C > A:T transitions at CpG sites were significantly more frequent in secondary than in primary GBMs [67]. The less specific pattern of *TP53* mutations in primary GBMs suggests a different molecular mechanism underlying the acquisition of *TP53* mutations in these subtypes.

Amplification of *MDM2* is present in < 10% of GBMs, and this event appears to be associated to primary GBMs with no *TP53* mutations [72]. Loss of p14 [ARF] expression has been observed

frequently in GBMs (76%), and correlated with homozygous deletion or promoter methylation of the p14 [ARF] gene [53]. Comparing the overall frequency of p14 [ARF] alterations between primary and secondary GBMs, no significant difference was observed, while p14 [ARF] promoter methylation was more frequent in secondary than primary GBMs [53]. The analysis of multiple biopsies from the same patients revealed p14 [ARF] methylation already in one-third of low-grade astrocytomas [53].

But, have all these data any prognostic value for GBMs? Although there is some discordance among different studies, promising data have already been gained. Hence, some studies showed no association between *TP53* status and outcome of GBM patients [73, 74] or between p53 score analyzed by IHC and patient survival [75]. Schmidt *et al.* [76] analyzed 97 GBM cases and found that the presence of *TP53* mutations was a favorable prognostic factor. In the same way, Ohgaki *et al.* [67] showed that the presence of *TP53* mutations was a favorable prognostic factor, and at the population level, univariate analysis revealed that the presence of these mutations was predictive of longer survival; however, age-adjusted multivariate analysis revealed no difference in survival between patients with and without *TP53* alterations.

El Hallani *et al.* [77] showed that the Pro/Pro genotype (a functional single nucleotide poly-morphism at codon 72 of *TP53* gene results in the presence of either proline (Pro) or arginine (Arg) in the amino acid sequence) is significantly over-represented in young patients with GBM (<45 years) (7 of 43 cases, 16.3%) compared to older patients (>45 years) (14 of 217, 6.5%) (*P*=0.05), whereas no difference of frequencies for Arg/Arg versus Arg/Pro between the two groups were observed. These data suggest a recessive effect of the Pro allele on the oncogenesis of GBM in young patients. This result is in line with previous reports showing consistent associations between the codon 72 polymorphism with age of onset in oral cancer, head and neck carcinomas, hereditary nonpolyposis colorectal cancer, and prostate cancer [78, 79]. The polymorphism described by El Hallani *et al*. [77] at *TP53* codon 72 is associated with age at onset of glioblastoma. In a study in 2009, Zawlik et al. (66) revealed that *TP53* codon 72 Pro allele was significantly associated with shorter survival among patients with GBMs carrying a *TP53* mutation (Arg/Pro or Pro/Pro), and among those treated with surgery plus radiother-apy (Arg/Pro).

Considering the association between mutations and treatments, recent studies have shown that the status of the *TP53* gene interferes with the effectiveness of treatment by DNA alkylating agent temozolomide (TMZ), the most effective chemotherapeutic for GBM. Blough *et al.* [80] related that GBM cell lines that did not express a functional p53 were significantly more sensitive to TMZ than cell lines with functionally intact wild-type p53 expression, while altered p53 expression or function had only minor effects on TMZ sensitivity in brain tumor initiating cells and tended to decrease sensitivity to TMZ.

2.1.2. Oligodendroglial and oligoastrocytic tumors

In contrast to diffuse astrocytomas, loss of 17p and *TP53* mutations are rare in oligodendroglial tumors (~10%) and mutually exclusive to 1p/19q deletion, a hallmark alteration in oligoden-drogliomas (~ 70%), while oligoastrocytomas frequently carry either *TP53* mutations (~40%)

or loss of 1p/19q (~45%), indicating that oligoastrocytomas are genetically monoclonal, and carry genetic alterations similar to either diffuse astrocytomas or oligodendrogliomas. Furthermore, G:C > A:T transitions at CpG sites are the most frequent *TP53* mutations in these tumors [81-83]. According to Muller *et al.* [84] oligoastrocytomas in the temporal lobe showed LOH on 1p and 19q less frequently (33%) than *TP53* mutations (45%). In contrast, oligoastrocytomas arising outside the temporal lobe demonstrated LOH on 1p and 19q in nearly 75% of the cases while *TP53* mutations were found in less than 20% [85].

Watanabe *et al.* [86] reported that genetic alterations in the p53 pathway are more frequent in anaplastic oligodendroglioma (50%) than in oligodendroglioma WHO grade II (21%), and showed that simultaneous disruption of the *RB1/CDK4/p16* ^{INK4a} */p15* ^{INK4b} and the *TP53/p14* ^{ARF} */MDM2* pathways occurs in 45% (9/20) of anaplastic oligodendrogliomas, suggesting that these phenomena contribute to their malignant phenotype. Anaplastic oligoastrocytoma typically exhibits the type and distribution of molecular lesions observed in oligoastrocytoma: loss of 1p/19q or *TP53* mutations [84].

A number of genetic alterations have been correlated with poorer response to chemotherapy or worse overall survival in anaplastic oligodendrogliomas. Ino *et al.* [87] suggested that a variety of relatively infrequent genetic alterations (*EGFR* gene amplification, 10q loss, *CDKN2A* homozygous deletion, *PTEN* mutation, and *TP53* mutation) were associated with worse prognosis. Interestingly, *TP53* mutation was associated with an improved likelihood of chemotherapeutic response but with a poor overall prognosis, since responses were not durable in the setting of *TP53* mutation.

Kim *et al.* [83] evaluated 413 tumors confirmed as low-grade diffuse gliomas WHO grade II (206 diffuse astrocytomas, 73 oligoastrocytomas, and 134 oligodendrogliomas) and observed that the median survival of patients with *TP53* mutation combined with *IDH1/2* mutation was significantly shorter than the observed in patients with 1p/19q loss combined with *IDH1/2* mutation (51.8 months vs. 58.7 months, respectively; P=0.0037). A Multivariate analysis with adjustment for age and treatment confirmed these results (P=0.0087) and also revealed that *TP53* mutation is a significant prognostic marker for shorter survival (P=0.0005) and 1p/19q loss for longer survival (P=0.0002).

2.1.3. Ependymal tumors

TP53 mutations were rarely reported in ependymal tumors by molecular analysis [88, 89]. However, p53 protein is identified in about 60% of ependymal tumors [90]. Shuangshoti *et al.* [91] suggested that the discrepancy may be due to expression of wild type p53 gene in tumor cells, alternative mechanisms of p53 gene inactivation or simply a cross-reaction of the antigen-antibody complex.

A number of studies have documented a correlation between p53 expression and tumor grade in ependymomas [90, 92, 93]. Sharma *et al.* [94] analyzed p53 protein expression in 119 ependymomas tumors (17 cases were of grade I, 54 of grade II and 48 of grade III) and observed its expression in only two cases of grade I tumors (11.5% and 6.4%). Five cases of grade II tumors showed p53 protein expression and this percentage of nuclear positivity was very low

(< 1.0%), while eighteen of 48 grade III tumors (37.5%) showed expression of p53 and mean positivity was 5.5%. Manasa *et al.* [95] reported 66% p53 positivity, performing p53 immuno-histochemical analysis in 54 samples of different grades and subtypes of ependymomas and observed that p53 indices were higher in grade II and grade III tumors (26.27 % and 26.08% respectively) as compared to subependymomas (grade I) (7.25%). However, p53 index of myxopapillary ependymoma (grade I) (26%) was similar to grade II and grade III tumors. But these values did not show statistical significance (P=0.2). Papillary ependymoma (grade II) showed p53 expression in 24% cells.

Some authors have advocated that p53 immunolabeling are important prognostic markers in ependymomas. Zamecnik *et al.* [96] found that p53 immunopositivity is the strongest indicator of aggressive tumor behavior and poor prognosis. Gaspar *et al.,* [88] studied the p53 pathway in primary intracranial childhood ependymomas and p53-mediated response to DNA-damage in two newly described ependymoma xenograft models. Their findings do not suggest a role of p53 genetic/epigenetic alterations in the tumorigenesis or progression of childhood ependymomas; however, radioresistance of these tumors might be due to alterations in p53-mediated growth arrest. Despite the lack of *TP53* mutations, immunocytochemical accumulation of p53 occurs, particularly in tumors with poor outcome. Moreover, the data concerning immunoexpression of the p53 protein indicate its usefulness in identification of more aggressive clones in ependymomas and its superior predictive value [94].

2.2. Embryonal tumors

2.2.1. Medulloblastoma

The genetic and genomic understanding of medulloblastoma (MB) has evolved dramatically in the past few years, but the role of p53 in MB pathogenesis has only initiated to be elucidated. Patients with LFS, caused by a germline mutation in p53, develop MB at a higher incidence than the general population [97, 98]. Similarly, p53 deficiency in mice in combination with mutations in other genes, including poly (ADP-ribose) polymerase (PARP), the cell cycle regulatory protein retinoblastoma (Rb), or the Sonic hedgehog (Shh) receptor Patched1 (Ptch1), greatly increases tumor incidence [99, 100], indicating that loss of p53 can promote MB tumorigenesis. However, in contrast with the high incidence of p53 mutations in most human tumors, the *TP53* gene is altered in <10% of sporadic human MB. Chromosome 17p, where *TP53* is located, is lost in 40% to 50% of sporadic MB tumors. However, it has been found that losses of 17p and p53 status are unrelated in MB [101, 102].

New support for a role for p53 in MB tumorigenesis came from a better understanding of heterogeneity underlying MB tumors. Recently, several groups were able to demonstrate that although morphologically similar, MBs could be divided into several subgroups on the basis of expression profiling [103, 104]. A consensus meeting resulted in the current molecular subclassification of MB into four subgroups: wingless (WNT), sonic hedgehog (SHH), group 3, and group 4 [105]. Hopefully, in the near future, this subclassification will be used to select targeted therapies and improve understanding of the behavior of this disease.

As observed for other CNS, reports detailing the prognostic impact of *TP53* mutations in MB offer conflicting conclusions. Pfaff *et al.* [106] reported that *TP53* mutations occur at low frequency in MBs but are overrepresented in the prognostically favorable subgroup featuring alterations in the Wnt pathway. In addition, because no correlation between *TP53* mutation status and patient outcome was observed in more than 300 patients, these authors concluded that *TP53* mutation is not a universal prognostic marker for MB. These results were supported by Lindsey *et al.* [107] in an independent and representative series of all major established clinical and molecular subtypes of MBs. Nevertheless, Gessi *et al.* [108] reported that *TP53* expression is associated with rapid disease progression and poor prognosis in patients with metastatic MB, with a statistically significant inverse correlation between *TP53* expression and patient survival.

A large whole-genome and exome sequencing efforts recently published by different groups revealed an additional, albeit small number, of *TP53* mutations in MB [109, 110]. These independent groups found *TP53* mutations enriched in the SHH group and associated with poor survival. Zhukova *et al.* [111] evaluated the association of *TP53* mutations, molecular groups, and survival in MBs patients and confirmed that *TP53* mutations are enriched among SHH MBs, in which they portend poor outcome and account for a large proportion of treatment failures in these patients.

Carvalho *et al.* [112] were the first to investigate the role of the *TP53* Arg72Pro SNP as a potential risk factor and/or prognostic marker of MB by performing a case–control analysis using a polymerase chain reaction-restriction fragment length polymorphism approach. The date suggested that, although there is no association between the *TP53* Arg72Pro SNP and MB risk, the Pro/Pro genotype is associated with shorter overall survival of patients submitted to adjuvant therapy.

Some researchers justify the p53 inactivation in MB tumors lacking *TP53* gene mutations through alternative mechanisms. Mendrysa *et al.* [113] supported MDM2 as an important contributor to the inhibition of p53 in SHH-driven MB tumorigenesis. In cerebellar development, *MDM2* is required to inhibit p53-mediated apoptosis in granular neuronal precursors, the presumed cell of origin for MB tumors of the Shh subgroup, and *MDM2* deficiency potently restricts cerebellar tumorigenesis in Ptch1+/− mice, a model of human Shh-induced MB.

2.2.2. CNS primitive neuroectodermal tumors

The presence of *TP53* mutations have been identified in CNS primitive neuroectodermal tumors (PNETs), mainly in adult patients [114]. However, *TP53* mutations in PNET have also been occasionally reported in children [115, 116], but the overall incidence of somatic *TP53* mutation in pediatric CNS-PNET seems to be very low [115]. Gessi *et al.* [117] analyzed the clinicopathologic and molecular features of 12 cases of PNETs in adult patients. The p53 staining showed strong nuclear positivity (>20% of stained nuclei) in 9 cases, evidencing the presence of *TP53* mutations in these tumors. The use of single strand conformation polymorphism (SSCP) followed by sequencing of the *TP53* gene showed point mutations of this gene in 4 of these 9 cases, identifying 5 mutations in exons 4, 5, 7, and 8.

Although the presence of *TP53* mutations seems to mainly occur in adult s-PNETs, nuclear accumulation of p53 has been described to be frequent not only in adults but also in pediatric CNS-PNETs [118]. This observation led to the hypothesis that the p53 pathway is pivotal in CNS-PNET biology and can also be activated by mechanisms other than mutation.

Immunohistochemical staining for the p53 gene product is a good predictor of poor outcome in PNETs. Robert *et al.* [119] observed stained intensely for the p53 protein in 10 patients (n=40) with PNETs and 11 had weakly staining nuclei, while 19 specimens had no staining. The patients with specimens that stained intensely had a statistically significant decreased disease free survival (P=0. 03). Intense p53 immunostaining may predict a poor prognosis in PNETs of childhood [120], however, the significance of p53 in recurrent CNS PNETs is unknown.

2.2.3. Atypical teratoid/ Rhabdoid tumor

The role of p53 in atypical teratoid/ rhabdoid tumor (AT/RT) is also poorly understood. Cell lines established from malignant rhabdoid tumor (MRT) show overexpression of p53, without associated *TP53* gene mutations [120]. On the other hand, missense mutations in *TP53* were reported in 3/6 cases of non-CNS MRT [121]. Knockdown of *SMARCB1* in cell lines and animal models results in activation of p53 [122, 123]. Intriguingly, combined inactivation of *Smarcb1* and *TP53*, but not *Rb* or $p16^{ink4a}$, leads to accelerated development of MRT in mouse models [122, 124]. These data have led to the hypothesis that two successive hits involving *SMARCB1* and *TP53* may contribute to malignant transformation and tumor development. Venneti *et al.* [125] studied the expression of p53 and determined *TP53* mutational status in 36 AT/RT and 16 non-CNS MRT patients. They also studied the relationship of p53 expression with its regulators $p14^{ARF}$/*MDM2* in AT/RT and non-CNS MRT. p14 ^{ARF} expression was seen in many cases, which correlated positively with p53 and inversely with Mdm2 immunostaining in AT/RT, while *TP53* mutational analysis in 19/25 AT/RT and 8 in 11 non-CNS MRT cases showed point mutations in only 3 AT/RT cases, suggesting that p53 expression was driven mainly by p14 ^{ARF}.

2.3. Choroid plexus tumors

Choroid plexus tumors (CPT) are rare tumors, often occurring during childhood. Previous studies have shown high frequencies of germline *TP53* mutations in patients with CPT (44–100%) irrespective of family history [126, 127]. According to the latest clinical criteria for LFS, it is suggested that patients with CPT should be considered for *TP53* testing [128, 129]. In addition, somatic mutations of the *TP53* gene and subsequent accumulation of p53 protein have been described in up to 50 % of choroid plexus carcinomas (CPC) [130, 131].

The prognostic role of p53 in choroid plexus carcinomas has been recently demonstrated. Tabori *et al.* [131] studied 54 patients with CPTs, including CPC (n=36) and choroid plexus papilloma (CPP) (n=18), and demonstrated that patients with CPC who have low tumor total structural variation and absence of *TP53* dysfunction had a favorable prognosis and could be successfully treated without radiation therapy. Krzyzankova *et al.*, [132], investigated the role of p53 in the growth-inhibitory potential of a variety of anticancer agents in the immortalized

rodent choroid plexus epithelial cell line Z310 and observed that growth-inhibitory activity of vincristine, doxorubicin, carboplatin, etoposide, and TMZ was significantly impaired by silencing of *TP53*, showing the potential predictive role of p53 in choroid plexus carcinomas.

2.4. Meningiomas

Few studies have examined the *TP53* gene directly for mutations in meningiomas, [133-135], and these studies typically have not observed mutations in this gene, although rare mutants have been described, mainly associated with malignant histology [134, 136]. One group working on specimens from Korean patients has documented a rate of nearly 40% of p53 over-expressing meningiomas as having mutations, and observed that the mutation rate was associated with both histological grade and recurrence [137].

In contrast the low frequency of *TP53* point mutations, expression of p53 was found in 10% to 90% of meningiomas [138], but their role in pathogenesis is still uncertain. Studies suggested the involvement of the p53 pathway in meningioma development: the correlation of p53 protein expression with histological tumor grade and meningioma recurrence [139]; methylation of the *p14 ARF* gene in 8.6% of benign, 20% of atypical and in 50% of anaplastic meningiomas and loss of detectable Mdm2 protein in high grade meningiomas [140]; defective p53 response to gamma ray stress in meningioma cells [141]. In addition, the *NF2* protein product was reported to increase p53 stability through downregulation of Mdm2 levels in mouse fibroblast [142]. It follows that loss of NF2 may increase the likelihood of p53 suppression, thus decreasing tumor suppression activity and providing a possible mechanism for the involvement of the p53 pathway in meningiomas.

Many studies have examined benign and atypical/malignant meningiomas for over-expression of the p53 protein with diverse results: p53 over-expression has been reported in 0–10% of benign, 50–72.7% of atypical and 77–88.9% of anaplastic or malignant meningiomas [143]. Despite the differing rates, all of the studies are consistent, with atypical/malignant tumors showing higher rates of over-expression than benign meningiomas. However, studies on the biological significance of p53 over-expression are highly contradictory. While over-expression of p53 has been associated with recurrence in some studies [139, 144], no association has been found in others [133, 145]; and still other studies have suggested that expression of high levels of p53 may be protective against recurrence [146].

Terzi *et al.* [147] analyzed the immunohistochemical expression of Ki-67, p53, p21, p16, and *PTEN* proteins in 130 meningiomas (64 benign, 39 atypical, and 27 malignant meningiomas) using tissue microarray and demonstrated that Histological grade, p53, Ki-67 labeling indices, and overexpression of p16 were strongly associated with decreased event-free survival in univariate analysis and Ki-67 and p53 labeling indices are useful additional tools in discriminating atypical from benign or anaplastic meningiomas.

3. Epigenetic mechanisms in CNS tumors

Epigenetics is defined as mitotically heritable changes in gene expression that are not due to changes in the primary DNA sequence. The coordinated interaction of these changes regulates gene expression activity and several types of epigenetic marks work in concert to drive appropriate gene expression, like DNA methylation at CpG dinucleotides, covalent modifications of histone proteins, non-coding RNAs, and other complementary mechanisms controlling higher order chromatin organization within the cell nucleus. Epigenetic alterations have been recognized as important mechanisms in neoplastic transformation, malignant progression of cancer, and although epigenetic changes are somatically inheritable, they are reversible and hence may represent actionable targets for novel therapies [148, 149]

Epigenetic changes are often observed at the earliest stages of neoplasia within the altered tissue stem and progenitor cells. These observations have led to the epigenetic progenitor model [149]. This model explains that transformation to a malignant state occurs in three steps. First, there is an expansion of an epigenetically permissive population due to an essential early epigenetic disruption of stem/progenitor cells. Second, an initiating genetic alteration in an oncogene or tumor suppressor gene occurs. Finally, genetic and epigenetic plasticity resulting in an enhanced ability to stably evolve the phenotype is observed. An important difference to the clonal genetic model is that the epigenetic 'hits' occur early, and are necessary to create an appropriate expansion of a polyclonal population, that is the cellular substrate for subsequent genetic alterations and transformation [150].

To better understand the multiple cellular pathways involved in their development, establishment markers of resistance to traditional therapies, and contribution to the development of targeted therapies, a comprehensive appreciation of the integrated genomics and epigenomics of CNS tumors is needed [151].

3.1. DNA methylation of gene *TP53*

Hypermethylation of promoters usually occurs at CpG islands. Methylation of *TP53* was reported as a mechanism for its inactivation in neoplasias, such as acute lymphoblastic leukemia, multiple myeloma, malignant glioma cells, and brain metastases of solid tumors [152]. Since the promoter region of *TP53* does not contain a classic CpG island, methylation of one or two sites may produce a proportionately greater effect in downregulation of transcription compared to a tumor suppressor gene with a classic CpG island in the promoter [153]. The *TP53* promoter region has been sequenced and basal promoter activity localized to an 85 bp region (nucleotide 760–844) that is indispensable for full promoter activity and the *TP53* promoter has putative binding sites for transcriptional factors [154]. Schroeder and Mass [155] have shown that methylation in the promoter region of the p53 gene reduces reporter gene activity. They found down-regulation of p53 in cultured cells transfected with a plasmid incorporating a *TP53* promoter containing methylated CpG dinucleotides. Furthermore, this region has been shown to be methylated in several cancers [156].

Analyses of methylation of *TP53* promoter region are controversial. While some researchers reported low frequencies of *TP53* methylation in neuroblastic tumors (0/44), astrocytomas (2/24, 8%), GBM (1/43, 2%) [157], oligodendroglial tumors (0/41) and ependymomas (0/7) [158], other authors observed a higher frequency [159, 160]. The reason for this discrepancy remains to be clarified.

Amatya *et al.* [159] assessed whether promoter methylation was present in cells of six malignant gliomas and whether there is an association with reduced expression of *TP53* mRNA and protein. They also assessed the frequencies of disruption of the p53/p14 ^{ARF} pathway in 49 low-grade astrocytomas (40 fibrillary astrocytomas and 9 gemistocytic astrocytomas), 42 oligodendrogliomas and 18 oligoastrocytomas. The Methylation-specific PCR (MS-PCR) revealed methylation of the promoter region of the *TP53* gene in three (U87MG, LNT-229, T98G) out of six malignant glioma cell lines. Real time RT-PCR revealed that two malignant glioma cell lines (U87MG and T98G) led to up-regulated expression of *TP53* mRNA and protein after treatment with 5-aza-2'-deoxycytidine (5-aza-dC, an epigenetic modifier that results in DNA demethylation), suggesting that promoter methylation is associated with reduced expression in some malignant glioma cells. *TP53* promoter methylation in primary tissue of low-grade gliomas was observed in 29/48 (60%) low-grade astrocytomas, 11/18 (61%) oligoastrocytomas, and 31/42 (74%) oligodendrogliomas, while promoter methylation of the p14 ^{ARF} was detected by MS-PCR in 5/49 (10%) low-grade astrocytomas, 7/18 (39%) oligoastrocytomas, and 15/41 (37%) oligodendrogliomas. Briefly, alterations of at least one of *TP53* promoter methylation, p14 ^{ARF} promoter methylation, and *TP53* mutations were found in 43/49 (88%) of low-grade astrocytomas, 15/18 (83%) of oligoastrocytomas, and 35/42 (83%) oligodendrogliomas, suggesting that disruption of the p53/p14 ^{ARF} pathway is frequent in all histological types of low-grade glioma.

Almeida *et al.* [160] evaluated the promoter hypermethylation profile of the *TP53* gene in 90 extra-axial brain tumors (48 meningiomas, 23 schwannomas and 19 metastases) using MS-PCR and sequencing. The group showed that the methylation of the *TP53* gene is an important event associated with extra-axial brain tumors, since 37.5% of meningiomas, 30% of schwannomas and 52.6% of metastases were hypermethylated. When tumor grade was compared, 35.3% of benign tumors and 48% of malignant tumors were methylated, and these results suggested that *TP53* methylation can be involved in the progression of these tumors.

3.2. The new insights of MicroRNAs/*TP53* in cancer

MicroRNAs (miRNA) are a large class of small, non-coding RNAs, 21 – 28 nucleotides long, produced naturally in cells after being cut into segments from larger strands of RNA by the enzyme Dicer. They function by binding to complementary sites on the 3'-untranslated region (3'-UTR) of genes and promoting the recruitment of protein complexes responsible for impairing translation and/or decreasing the stability of mRNA [161, 162]. A specific miRNA may simultaneously regulate multiple targets, thereby enabling complex changes in protein expression profiles. Furthermore, a single target can be regulated by multiple miRNAs, and upstream regulation of a given miRNA can involve multiple regulators at different steps of miRNA biogenesis. Thus, miRNAs take part in complex regulatory networks that may

influence almost every cellular process [163]. Currently, 1, 048 human microRNAs are known to modulate approximately 3 % of all genes and up to 30 % of protein-coding genes. Vital for protein expression, microRNAs are integrally associated with both normal and abnormal biological processes [164].

miRNAs play important roles in the regulation of normal gene expression at developmental timing, cell proliferation and apoptosis [165]. As these processes are altered in cancer cells, there are in literature several studies that were undertaken to provide evidence for an involvement of miRNAs in cancer formation. miRNA-encoding genes as well as mRNA-encoding genes have been meanwhile classified as oncogenic or tumor suppressive genes according to their function in cellular transformation and expression in tumors [166, 167]. Furthermore, tumor cells seem to undergo a general loss of miRNA expression, and forced reduction of global miRNA expression promotes transformation [168]. Interestingly, miRNAs cluster within fragiles sites and other genomic regions frequently altered in cancers [169]. Because of their role in tumor formation, miRNAs may be very useful for the classification, diagnosis, prognosis, and therapy of malignancies [166, 167].

Profiling miRNA provides an attractive, novel, and non-invasive biomarker for tumor diagnosis and prognosis. Molecular biology techniques, such as Northern blot, RNase protection assay, and primer extension assay can measure expression of a miRNA. The small size of miRNAs initially hampered polymerase chain reaction-based methods. However, PCR-based techniques have become very popular since the development of adaptor-mediated quantitative real-time PCR (qRT-PCR) due to their high sensitivity [170]. Microarray techniques are widely used to comprehensively assay the entire miRNome (the global miRNA expression profile) in tissues or in cell lines [171]. In addition to microarray and qRT-PCR, miRNomes are obtained by *in situ* hybridization [172] and serial analysis of gene expression adapted for small RNAs [173]. Overall, these technical improvements are expected to greatly widen the repertoire of miRNAs in a variety of biological systems.

p53 is a transcription factor, so transactivates or represses many protein-encoding genes and this underlies much of its tumor suppressor function. Recently, it has been reported that p53 directly transactivates specifics miRNAs [174]. miRNA have also been shown to target p53 and/or components of p53 regulatory pathways affecting its activities directly and/or indirectly [175, 176].

Several reports shed light on the involvement of miRNAs in the p53 pathway. He *et al.* [177] profiled miRNA gene expression in wild-type (wt) and p53-deficient cells and found that the miR34s (miR-34 gene family, including miR-34a, b and c) was among the most upregulated in wt p53 cells. In addition, Luan *et al.* [178] analyzed the expression levels of miR-34a in human glioma cell lines (U251, A172 and SHG-44) using real time quantitative PCR and compared with that observed in normal brain and determined its role in cell proliferation, cycle distribution, apoptosis and capabilities of in vitro migration and invasion of p53-mutant glioma cells. The results showed that miR-34a is remarkably reduced in p53-mutant glioma cell line U251, that had a mutation of codon 273 (CGT/CAT; Arg/His) in exon 8 than other p53-wild glioma cell lines A172, SHG-44 and normal brains.

miR34s are induced after genotoxic stress in a p53-dependent manner *in vitro* and *in vivo*. miR-34b and-34c are clustered at chromosome 11, whereas miR-34a is located in a separate genomic locus. p53 directly activates both pri-miRNAs. The miR-34s seem to be critical downstream effectors of p53, as ectopic expression of the miR-34s recapitulate the phenotype of p53 activation. The miR-34s promotes repression of several direct targets, such as Bcl-2, Cdk4, hepatocyte growth factor receptor (MET), and other, resulting in cell cycle arrest, apoptosis, and senescence [179] (Figure 3). Several other laboratories corroborated the finding that miR-34s are critical components of the p53 network [180-182]. Taken together, these results support a pivotal downstream role of miRNAs in the regulation of the p53 pathway.
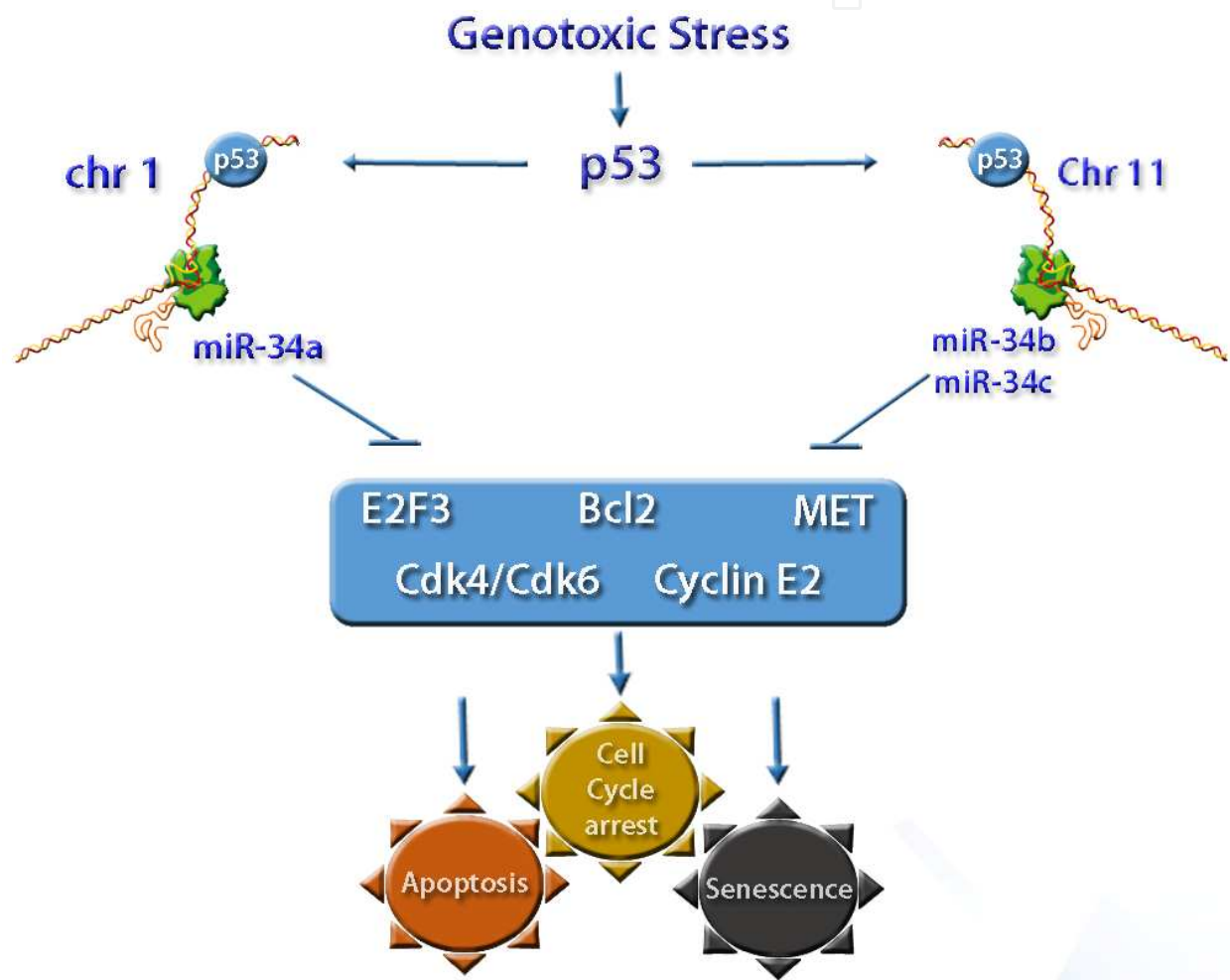

Figure 3. Representation of p53 and the miR-34 family interactions. The p53 protein stimulates the transcription of miR34s, which inhibits oncoproteins and leads to cell senescence, apoptosis and cell cycle arrest.

As cell cycle arrest, senescence, and apoptosis are tumor suppressive mechanisms, the inactivation of members of the miR-34 family, which induce these cellular responses, may be a selective advantage for cancer cells. Besides decreased expression of MiR-34 due to inactivating mutations of p53, the miR-34 encoding genes themselves may be targets for mutational or epigenetic inactivation in cancer. For example, loss of miR34a expression was observed in

neuroblastoma, which may be due to the relatively common deletion of a region on chromosome 1p36, which encompasses miR-34a [183]. However, the mechanisms leading to decreased expression of miR-34s require further exploration.

Other miRNAs may be important in the p53 network. miR-30c, -103, -26a, -107, and-182 were induced clearly, although less robustly, upon DNA damage in a p53-dependent manner [181]. In another approach, the searching for p53-binding elements in DNA sequences near miRNAs identified miR-129 as a good candidate for regulation by p53 [184]. miR-125b, a brain-enriched microRNA, was identified as a bona fide negative regulator of p53 in both zebrafish and humans [185]. Recently, Hu *et al.* [186] showed that miR-504 directly represses p53 expression and function in human cell lines.

Since recent studies have indicated that p53 enters into miRNA world [187], some researchers provided important insights into the central roles of miRNAs in a well-known tumor suppressor network, the p53 pathway, which may provide a route to therapeutic miRNA intervention in CNS tumors. Shyamal *et al.* [188] were the first to demonstrate that miR-34a directly targets the *MAGE-A* family of oncogenes, disengaging p53 from *MAGE-A*–mediated repression. The group demonstrated that miR-34a directly targets the 3′UTR of *MAGE-A* genes and decreases MAGE-A protein levels in medulloblastoma cell lines. This decreasing in *MAGE-A* results in a concomitant increasing in p53 and its associated transcriptional targets, p21/ WAF1/CIP1 and, importantly, miR-34a. This establishes a positive feedback loop where miR-34a is not only induced by p53 but increases p53 mRNA and protein levels through the modulation of *MAGE-A* genes and a consequence of this mechanism is that sensitizes medulloblastoma cells to chemotherapeutic agents via delayed G2/M progression and increased apoptosis.

Recently, Suh *et al.* [189] identified two miRNAs (miR-25 and-32) as p53-repressed miRNAs in glioblastoma multiforme cells through p53-dependent negative regulation of their transcriptional regulators, E2F1 and MYC. The study provided compelling evidence that expression of these miRNAs causes tumor suppression through mechanisms that lead to accumulation of p53 protein, by directly targeting Mdm2 and TSC1, leading to inhibition of cellular proliferation through cell cycle arrest. Thus, there is a recurrent autoregulatory circuit involving expression of p53, E2F1, and MYC to regulate the expression of miR-25 and-32, which are miRNAs that, in turn, control p53 accumulation. Significantly, overexpression of transfected miR-25 and-32 in cells of GBM inhibited growth of these cells in mouse brain *in vivo*. The results define miR-25 and-32 as positive regulators of p53, underscoring their role in tumorigenesis in glioblastoma.

4. The cancer stem cell model

Until a few years ago, the brain was thought to lack a stem cell population, but actually, it is now known that there are two regions of the adult human brain that contain neural stem cells (NSCs) (a group of self-renewing cells in the nervous system that can generate both neurons

and glia): the dentate gyrus of the hippocampus and the subventricular zone. NSCs can form neurons, astrocytes and oligodendrocytes *in vitro*, although their normal physiological role in the adult human brain is disputed [190].

With the accumulation of knowledge concerning the stem cell and the mechanisms regulating their behaviour, it was noted that many of the characteristics of stem cells were also present in cancer. These findings reinforce the "cancer stem cell model", which states that the cellular heterogeneity within the tumor is ascribed entirely to the differentiating tumor cells that derive from the cancer stem cells (CSCs), that can be defined as cells that possesses the capacity to self-renew and to originate the heterogeneous lineages of cancer cells that comprise the tumor. The term ''tumor-initiating cell'' also has been used to describe a cell with the potential to initiate a tumor. This term are essentially functionally equivalent to CSCs if it is used to refer to the subclones of cells within an established tumor that gives rise to a new tumor when transplanted [190-192].

CSCs were first observed by John Dick's group in acute myeloid leukemia and posteriorly other researchers reported CSCs in solid tumors, including those formed by breast, colon, prostate, pancreatic, lung, liver and brain [193-196], Subsequently there has been a large amount of work to identify the cancer stem cell population, and to study its role in progression of disease and resistance to treatment, allowing many experimental therapies targeting cancer stem cells can be developed and tested in preclinical models [190, 195, 196].

CSCs have been isolated from a wide range of CNS neoplasms, including adult and pediatric, anaplastic oligodendrogliomas and malignant medulloblastomas [197]. For gliomas, several researchers isolated brain tumor stem cells (BTSC) from primary tumors based in the ability to form neurospheres NSCs do and other criteria: ability to be serially transplanted; unique ability to engraft; ability to recapitulate the tumor of origin morphologically and immuno-phenotypically in xenografts [198].

4.1. P53 role in neuronal and brain tumor stem cell

In the ependymal cell lining of the lateral ventricle wall as well as most cells of the subventricular zone, including astrocytes and progenitors, abundance of nuclear p53 is evident and in agreement with the down-regulation of p53 in differentiating cells observed during embryogenesis. The nuclear p53 immunoreactivity is absent or found at low levels in the majority of the mature brain, including differentiating cells in the rostral migratory stream, suggesting that p53 is preferentially expressed in neural precursors [113]. Several studies show the important role(s) of p53 in the regulation of mammary [199], hematopoietic [200], embryonic and neuronal stem cells [201] by regulating self-renewal, symmetric division, quiescence, survival, and proliferation.

Meletis *et al.* [202] demonstrated that *TP53* is expressed in the neural stem cell lineage in the adult brain and negatively regulates proliferation and survival, and thereby self-renewal, of neural stem cells. Analyses of the neural stem cell transcriptome identified the dysregulation of several cell cycle regulators in the absence of p53, most notably a pronounced downregu-

lation of p21 expression. These data reinforce the p53 role as a suppressor of tissue and cancer stem cell self-renewal.

Armesilla-Diaz *et al.* [203] demonstrated that p53 controls the chromosomal stability, proliferation and differentiation patterns of embryonic mouse olfactory bulb stem cells. The group reported that the absence of this protein increases the number of neurosphere-forming cells and the proliferation of these stem cells, and observed that differentiation of p53 knockout-derived neurospheres was biased toward neuronal precursors. Moreover, the relevance of p53 in maintaining chromosomal stability in response to genotoxic insult was demonstrated, and additionally, the results showed that neurosphere stem cells are highly resistant to long-term epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) deprivation in a p53-independent fashion, and they preserve their differentiation potential.

While the role of p53 in apoptosis of neuronal cells was well elucidated [202, 204, 205], its function in astrocytes, oligodendrocytes and their precursors is poorly understood. Oligodendrocyte precursors cultured *in vitro* can undergo p53-dependent differentiation although the cells appear to have a low basal level of p53 expression [206]. Both oligodendrocytes and astrocytes can undergo apoptosis following infection with an adenovirus expressing p53 [207].

CSCs exhibit genetic or chromosomal alterations in addition to aberrant differentiation properties, unlike the normal stem cells [208]. It is important to highlight the fundamental differences between normal stem cells and CSCs. The first are known for the vigilance with which their proliferation is controlled and for the care with which their genomic integrity is maintained, while CSCs lack such ability [209]. The cell-of-origin of CSCs remains elusive, however evidences indicate that CSCs may originate from malignant transformation of normal stem cells, because of the perennial nature and high proliferative potential characteristics of stem cells. Some studies have shown that oncogene activation or tumor suppressor gene inactivation increased the frequency of tumor formation in primitive nestin-expressing cells but not in the more differentiated glial fibrillary acidic protein (GFAP)-expressing astrocytes [210, 211] while other researches indicate that differentiated astrocytes and NSCs may be equally permissive to transformation when genomic alterations are introduced [212].

The role of p53 in BTSC has not been well established, however based on current understanding of its function in neural precursors available in the literature, several hypotheses may be develop. First, loss of p53 may increase the self-renewal and proliferation of neural stem and/or lineage-restricted progenitors, thereby expanding the pool of cells available for additional mutations in specifics oncogenes. Another hypothesis is that, depending on cellular context, p53 can both inhibit or promote cell differentiation, as well as influence cell fate decisions, so the differentiation program of neural precursors can be changed by p53 mutation. Lastly, accumulated evidences support a role for p53 in the suppression of cell migration, although much focus on p53 is directed at its growth inhibitory properties [213]. The neurogenic niche has been shown to be important for the maintenance of stem cells in an undifferentiated state, and the premature exit of NSCs from the neurogenic niche may alter their capacity for tissue invasion or differentiation program in the absence of p53 [214].

The number of studies concerning the cellular, molecular, and environmental factors that regulate p53 function in NSCs has increased drastically and brought a better understanding of these factors, and together with the advances in molecular biology techniques, provided much valuable information about the role of p53 in BTSCs. This scenario stimulates future studies exploring the significance of p53 alterations for prognosis and prediction of treatment response that would help development of individual treatment strategies as well as help clarifying the clinical importance of cancer stem cell biology.

5. p53-based gene therapy: GBMs as an example

Malignant tumors within the brain remain a therapeutic challenge, but current strategies tested in animal models as well as in the clinics have shown promising results. The rapid progress in knowledge of the p53 pathway have led to many different approaches to p53 based cancer therapy as mentioned previously and the field has excited great interest both academically and commercially [215]. The long awaited molecular treatment of GBM and other CNS tumors and utilization of knowledge surrounding p53 may then be foreseeable goals in the future. It will also be important and likely therapeutically be effective to combine gene therapy with other therapeutic modalities, including the standards-of-care [216].

Standard treatment of care for GBM, for example, consists of surgical resection, followed by radiotherapy and chemotherapy [217, 218]. Despite significant advances in current treatment approaches, including the gamma knife (radiation) and TMZ (chemotherapy) [217, 219], GBM continues to present a poor prognosis, with median survival still remains less than 15 months. It is important to remember that GBMs are the most common and least curable among CNS tumors [220]. Moreover, for this type of tumor, complete resection is practically impossible due to its diffuse nature and the proximity of the tumor to vital brain structures. Moreover, it often recurs in an area close to the original resection cavity [221]. The intrinsic resistance of glioblastoma cells to radiotherapy and chemotherapy confers another therapeutic challenge of this disease [222]. On the other hand it has been reported that invading GBM cells, which give rise to recurrences, are resistant to cytotoxic therapies due to the constitutive activation of antiapoptotic signaling pathways [221]. Novel therapeutic approaches and adjuvants to be employed in combination with standard therapeutic strategies are sorely needed for GBM patients, because although isolated traditional therapies allow an increase in the quality of life and survival of these patients, they are not curative and long-term survival is very rare [221, 223, 224].

Gene therapy for CNS tumors is evolving every year, especially for GBM, with the ultimate goal being specific delivery of therapeutic genes or oncolytic viruses to eliminate the tumor. Besides results in cell death, also enhanced immune responses to tumor antigens and disruption of the tumor microenvironment [216]. A variety of gene therapy strategies has been examined in GBM preclinical models and clinical trials and includes the use of selective replication-competent oncolytic viruses, non-replicating viral vectors or normal adult stem/

progenitor cells for the delivery of immunostimulatory genes, cytotoxic genes and genes modulating the tumor microenvironment [216].

The fact that p53 pathway is activated in tumor cells, but not in normal cells, provides a potentially important therapeutic selectivity, indifferent of which signal in the tumor cells activates p53 following its restoration [225]. In this context, the evidences that tumor cells, but not normal cells, have a cellular environment that activates the p53 pathway would create a setting of an advantageous therapeutic index, whose main objective is the development of interventions that selectively kill tumor cells instead normal cells [225].

Different approaches to achieve this goal are already in various stages of development and a diversity of small druglike molecules targeting the p53 system have been developed and several are now in clinical trials. Of critical importance has been the development of: agents which can increase active p53 in tumor cells by interfering with the p53–MDM2 interaction are therefore considered to have therapeutic utility in sensitizing tumor cells for chemo-or radiotherapy, such as the Nutlins [226, 227]; molecules that activate p53 via direct interaction with p53 itself, as PRIMA-1, of which there is evidence of induction of expression of mediators of p53-dependent apoptosis such as Puma, Noxa, and Bax in cells with mutant p53 [228, 229]; small molecules activating p53 family members in a p53 mutant or deficient background; molecules activating p53 by inhibiting class III histone deacetylases, nuclear export, transcriptional and nucleolar distuption. These screens in combination with RNAi based approaches are of utmost importance for the discovery of new targets for therapy in the p53 pathway [215].

Transfection of wild-type p53 in order to normalize function in mutant p53-containing tumors has been a long-pursued goal of gene therapy. Mercer *et al.* [230] initially demonstrated that plasmid-mediated transfection of the p53 gene is capable of suppressing cell growth in gliomas by inhibition of G0/G1 progression into S phase. Kock *et al.* [231] and Gomez-Manzano *et al.* [232] were among the first to demonstrate that delivering the p53 gene using an adenovirus vector (Ad-p53) resulted in high levels of apoptosis in glioma cell lines, by elevation of the levels of the p21 (cell cycle-related) and Bax (apoptosis-related) proteins. Frederick *et al.*[233] undertook a phase I trial of Ad-p53 in the treatment of patients with recurrent malignant gliomas with the purpose of determine the clinical toxicity of Ad-p53 and obtain molecular information regarding the expression and distribution of the p53 protein after intratumoral treatment of human gliomas with Ad-p53. Thus, their results conclude that Intratumoral injection of Ad-p53 allowed the exogenous transfer of the p53 gene and expression of functional p53 protein, with minimaltoxicity observed.

To the generation of an effective systemic anti-tumor immune response, it is necessary the development of strategies that promote the GBM tumor cell death, which is essential not only to kill tumor cells and reduce tumor burden, but also to induce the release of inflammatory molecules from dying tumor cells [234]. Drug combinations have been developed to selectively kill cancer cells that lack p53 function while protecting normal cells. The potential to explore the defective checkpoint status of cells with inactive *TP53* genes has also been largely recognized and in part stimulated the search of drugs that can inhibit PLK1, AURKB, and other proteins that regulate the G 2 /M checkpoint [235]. Shchors et al. [236] used a preclinical model of GBM in combination with a switchable p53 allele to model the therapeutic effect of p53

pathway restoration. It was observed that the therapeutic efficacy of p53 pathway restoration was greatly influenced by both the initial mechanism of p53 pathway-inactivating mutation and the temporal manner in which the selective pressure elicited by p53 pathway restoration was applied. Their results suggested that intermittent dosing regimens of drugs that restore wild-type tumor-suppressor function onto mutant, inactive p53 proteins will prove to be more efficacious than traditional chronic dosing by similarly reducing adaptive resistance.

This topic focused on GBM because of its poor prognosis and the target for most clinical trials. However, it is important to recognize that there are many other brain tumors which are also targets for gene therapy. Recently, Kunkele *et al.* [227] observed that targeting the p53-MDM2 complex using nutlin-3 significantly reduced cell viability and induced either apoptosis or cell cycle arrest and expression of the p53 target gene p21 in 4 of 6 human medulloblastomas cell lines. However, UW-228 and DAOY cells harboring *TP53* mutations were almost unaffected by nutlin-3, showing that the mutational status of the gene interfere in the efficacy of the treatment. MDM2 knockdown in medulloblastoma cells by siRNA mimicked nutlin-3 treatment, whereas expression of dominant negative p53 abrogated nutlin-3 effects. Oral nutlin-3 treatment of mice with established medulloblastoma xenografts inhibited tumor growth and significantly increased survival. Hence, the authors suggested that inhibition of the MDM2-p53 interaction with nutlin-3 is a promising therapeutic option for medulloblastomas with functional p53 that should be further evaluated in clinical trials.

6. Conclusion

After a detailed review of the literature about the role of the *TP53* gene in the genesis and development of CNS tumors we can conclude that both genetic and epigenetic alterations that inactivate this gene are directly related to these phenomena in specific histopathological tumors. In addition, several studies have investigated the predictive value of *TP53* mutation status and have shown that specific types of genetic mutations can alter the function and expression of p53, influencing tumor response to treatment and patient outcome, revealing thus to be a useful prognostic tool. Genetic alterations in the p53 pathway are early events in the molecular pathogenesis of diffuse astrocytoma and the highest frequencies of allelic loss and/or mutation of *TP53* gene are mostly seen in gliomas, and are a genetic hallmark of: low-grade diffuse astrocytomas (> 60%), mainly in gemistocytic astrocytomas that carry *TP53* mutations in up to 80% of the cases; anaplastic astrocytomas (40-70%); secondary glioblastoma (>65%); oligoastrocytomas (~40%). Genetic *TP53* mutations are rarely found or seen less frequently in other CNS tumors, however, some studies have shown that some changes have found important prognostic value. Recently, our group investigated the presence of numerical aberrations of chromosome 17 and *TP53* in 5 subjects with brain metastasis from breast cancer using dual-color fluorescence in situ hybridization experiments. Deletion of *TP53* was the most frequent alteration observed, suggesting that if this alteration is present in the primary tumors, breast tumors with loss of *TP53* copies have a poorer prognosis and a higher chance for metastasis [237].

Epigenetic events in *TP53* gene has been increasingly recognized as an alternative mechanism for inactivation of function of a tumor suppressor gene. Although less frequently, *TP53* epigenetic abnormalities has been found in CNS tumors and several reports shed light on the involvement of mainly DNA methylation and miRNAs in the p53 pathway, suggesting that this process can be involved in the genesis and progression of these tumors. Clearly, additional studies can provide important insights into the central roles of miRNAs in the p53 pathway, as well as *TP53* promoter methylation, which may provide a route to therapeutic intervention in CNS tumors.

Due the difficulty to the use of traditional therapeutic modalities such as chemotherapy and radiotherapy in the CNS tumors, especially in high grade tumors, such as glioblastomas, , it is expected that in a near future molecular treatment that could be obtain more effective control of disease progression will be used, resulting in an improved clinical course of these patients. Over the years, with the increasing advances of molecular biology techniques, much information has been obtained on the role of p53 in carcinogenesis. Because of the critical role p53 plays in a variety of cancers, a diversity of approaches have been undertaken to target p53 and its altered signaling pathways. Different drugs targeting the p53 system in order to activate the p53 pathway have been developed and several are now in clinical trials, and have shown promising results.

Nomenclature

AT/RT	Atypical Teratoid/ Rhabdoid Tumor
bFGF	Basic fibroblast growth factor
BCL2	B-cell CLL/Lymphoma 2
BTSC	Brain tumor stem cells
CSC	Cancer stem cells
CDK4	Cyclin-dependent kinase 4
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CNS	Central nervous systems
CHD5	Chromodomain helicase DNA binding protein 5
CPC	Choroid plexus carcinomas
CPP	Choroid plexus papilloma
CPT	Choroid plexus tumors
EGF	Epidermal growth factor
FISH	Fluorescence in Situ Hybridization
GFAP	Glial fibrillary acidic protein
GBM	Glioblastoma

IDH1	Isocitrate dehydrogenase 1 (NADP+)
IDH2	Iisocitrate dehydrogenase 2 (NADP+)
IHC	Immunohistochemistry
IARC	International Agency for Research on Cancer
LFS	Li-Fraumeni syndromes
LFL	Li-Fraumeni-like syndromes
LOH	Loss of heterozygosity
MRT	Malignant Rhabdoid Tumor
MDM2	MDM2 oncogene, E3 ubiquitin protein ligase
MDM4	Mdm4 p53 binding protein homolog
MB	Medulloblastoma
MET	Met proto-oncogene
MS-PCR	Methylation-specific PCR
miRNA	MicroRNAs
MYC	v-myc avian myelocytomatosis viral oncogene homolog
HNSCC	Head and Neck squamous cell carcinoma
NES	Nuclear Exclusion Domain
NLS	Nuclear Localization Domain
NSC	Neural stem cell
NF1	Neurofibromin 1
P14^{ARF}	Cyclin-dependent kinase inhibitor 2A (encoding p14)
P19^{ARF}	Cyclin-dependent kinase inhibitor 2A (encoding p19)
p15 ^{INK4b}	Cyclin-dependent kinase inhibitor 2A (encoding p15)
p16 ^{INK4a}	Cyclin-dependent kinase inhibitor 2A (encoding p16)
PTCH1	Patched homolog 1
PNET	Primitive neuroectodermal tumor
PTEN	Phosphatase and tensin homolog
qRT-PCR	Quantitative real-time PCR
RB1	Retinoblastoma 1
siRNA	small interfering RNA
SMARCB1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1
SSCP	Single strand conformation polymorphism
SHH	Sonic hedgehog
TMZ	Temozolomide

TP53	Tumor protein p53
TSC1	Tuberous Sclerosis 1
WHO	World Health Organization
WT	Wild-type
WNT	Wingless
PXA	Xanthoastrocytoma pleomorphic

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