CDKN2A/B Homozygous Deletions in Astrocytomas

Subjects: Oncology

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The *CDKN2A* and *CDKN2B* genes are located on the short arm of chromosome 9. *CDKN2A* encodes for two proteins, p14 and p16, and *CDKN2B* encodes for p15. These proteins regulate cell growth and angiogenesis. Interpreting the impact of *CDKN2A/B* alterations on astrocytoma prognosis is complicated by the changes in tumour classification and a lack of uniform standards for testing *CDKN2A/B*. While the prognostic impact of *CDKN2A/B* HD is established, the role of different *CDKN2A/B* alterations—heterozygous deletions (HeD), point mutations, and promoter methylation—is less clear. Consequently, how these alternations should be incorporated into patient management remains controversial.

Keywords: CDKN2A/B alterations ; CDKN2A/B homozygous deletions ; temozolomide

1. Introduction

In 2016, the WHO Classification of Tumours of the Central Nervous System (revised 4th edition) incorporated *isocitrate dehydrogenase 1* and *2* (*IDH1/2*) mutation status into the classification of diffuse gliomas [1]. IDH1/2 mutations (point mutations in either IDH1 codon R132 or IDH2 codon R172) lead to IDH dysfunction, which converts alpha-ketoglutarate to R-2-hydroxygluterate, driving oncogenesis and global epigenetic changes [1][2][3][4]. The WHO Classification in 2016 further stratified diffuse IDH-mutant gliomas into oligodendrogliomas and astrocytomas based on the respective presence or absence of chromosome 1p and 19q co-deletions (see **Figure 1**) [1].

IDH-mutant astrocytomas account for 80% of WHO grades 2 to 3 and 5% of high-grade astrocytomas [5][6]. When compared to IDH-wildtype glioblastomas, patients with IDH-mutant astrocytomas are younger at diagnosis (30–40 years vs. over 50 years). In addition, IDH-mutant astrocytomas have a more favourable prognosis compared to IDH-wildtype glioblastomas, even in high-grade cases, with grade 4 IDH-mutant astrocytomas having a median overall survival (OS) of 31 months, compared to IDH-wildtype glioblastomas with a median OS of 13 months [5][6]. Unfortunately, a proportion of IDH-mutant astrocytomas have poor outcomes similar to those of IDH-wildtype glioblastomas [7].

CDKN2A/B HD are identified in approximately 22% of IDH-mutant astrocytomas [8] and are thought to lead to the loss of cell cycle control and promote cell proliferation [9].

Due to the improved prognosis of tumours previously classified as IDH-mutant glioblastomas compared to IDH-wildtype glioblastomas, these have been reclassified as astrocytoma, IDH-mutant, CNS WHO grade 4 in the fifth edition of the WHO Classification of Tumours of the Central Nervous System (WHO CNS5, 2021). A hallmark of WHO CNS5 is the integration of molecular markers into tumour grading. As such, IDH-mutant astrocytomas with CDKN2A/B HD are classified as grade 4 tumours independent of morphologic features. Therefore, a diagnosis of astrocytoma, IDH mutant, or CNS WHO grade 4 requires either morphologic features of a glioblastoma, namely necrosis or microvascular proliferation, or homozygous deletion of CDKN2A and/or CDKN2B (see **Figure 1**) [1][10].

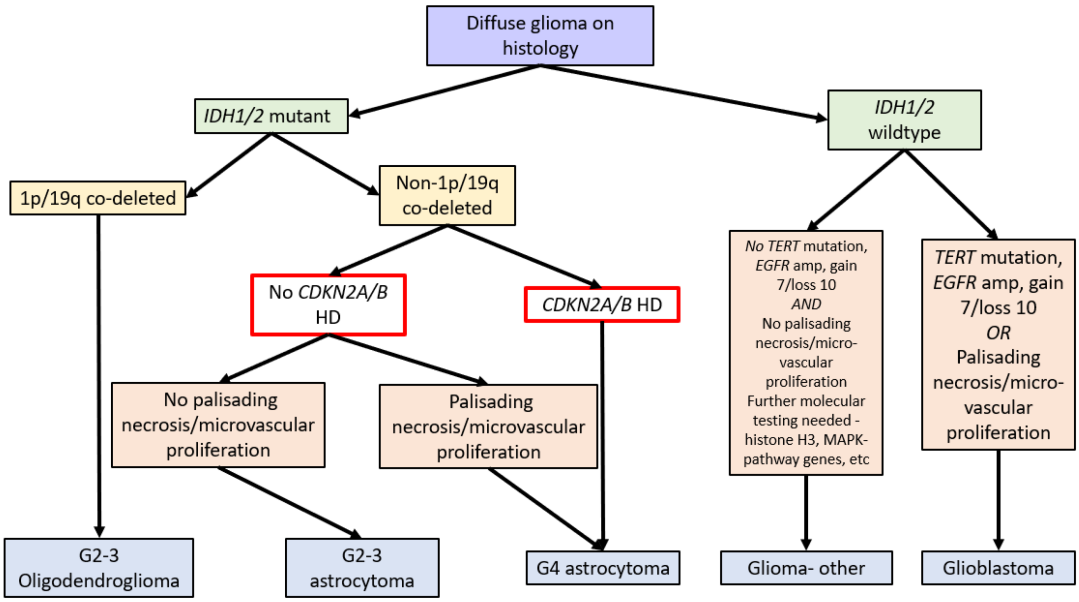

Figure 1. Flow diagram summarising the WHO Classification of CNS Tumours (fifth edition, 2021) [10] and the role of *CDKN2A/B* HD (red box). Note: gain 7/loss 10 refers to the gain of chromosome 7 and the loss of chromosome 10.

The significance of CDKN2A/B alterations in gliomas is difficult to assess in historical cohorts. Prior to 2016, many studies classified tumours based only on morphology. Consequently, tumours previously classified as astrocytomas on morphological grounds are likely to include tumours that are currently classified as astrocytoma, IDH-mutant, oligodendroglioma IDH-mutant, 1p/19q codeleted or glioblastoma, and IDH-wildtype [11][12][13]. Interpretation of the CDKN2A/B literature is further complicated by the multiple different techniques used to interrogate the CDKN2A and CDKN2B genes and whether one or both genes are interrogated. In addition, there is ambiguity concerning the significance of isolated CDKN2A or CDKN2B loss compared to loss of both CDKN2A and CDKN2B [14][15][16][17][18].

2. Normal Role of CDKN2A/B and Effect of Their Deletion

CDKN2A and *CDKN2B* are adjacent to each other on chromosome 9p21, with *CDKN2B* being 25 kilobases (kb) centromeric to *CDKN2A* [19]. These genes code for three proteins that suppress the oncogenic cyclin-dependent kinase (CDK) pathway. *CDKN2A* encodes for p14 and p16, and *CDKN2B* encodes for p15 [20]. Early cytogenetic studies identified recurrent loss of the short arm of chromosome 9 in glioma cell lines [21][22][23][24], many involving the 9p21 locus [22][25], which includes *CDKN2A* and *CDKN2B* [23][24].

The resultant loss of p14, p15, and p16 proteins from *CDKN2A/B* HD leads to dysregulation of the cell cycle and other parallel oncogenic processes (see **Figure 2**). Reflecting this, inactivation of *CDKN2A* function has been reported in a variety of other malignancies, including breast cancer, lung cancer, head and neck cancer, melanoma, and bladder cancer [9]. In normal cells, the retinoblastoma (Rb) protein prevents cell growth by binding to the transcription factor E2F, preventing its translocation into the nucleus. A complex formed by cyclin D and CDK4/6 can phosphorylate Rb, thereby releasing E2F and allowing translocation into the nucleus, leading to cell growth. The products of *CDKN2A/B*, p15 and p16, can directly inhibit the formation of the CDK4/6-cyclin D complex, maintaining the association between E2F and Rb [26][27][28] and preventing cell cycle progression. Another product of *CDKN2A* is p14, which acts on cyclin-CDK complexes indirectly by inhibiting MDM2. MDM2 tags p53, targeting it for ubiquitination and subsequent proteasomal degradation. 14 prevents MDM2 tagging, resulting in p53 stabilisation. This in turn promotes the cellular accumulation of the inhibitory protein p21, which blocks several cyclin-CDK complexes and promotes cell cycle arrest [29][30]. Due to these important functions of the protein products of *CDKN2A/B,* their deletion enhances oncogenic potential and leads to unregulated cellular proliferation (see **Figure 2**) [29][30].

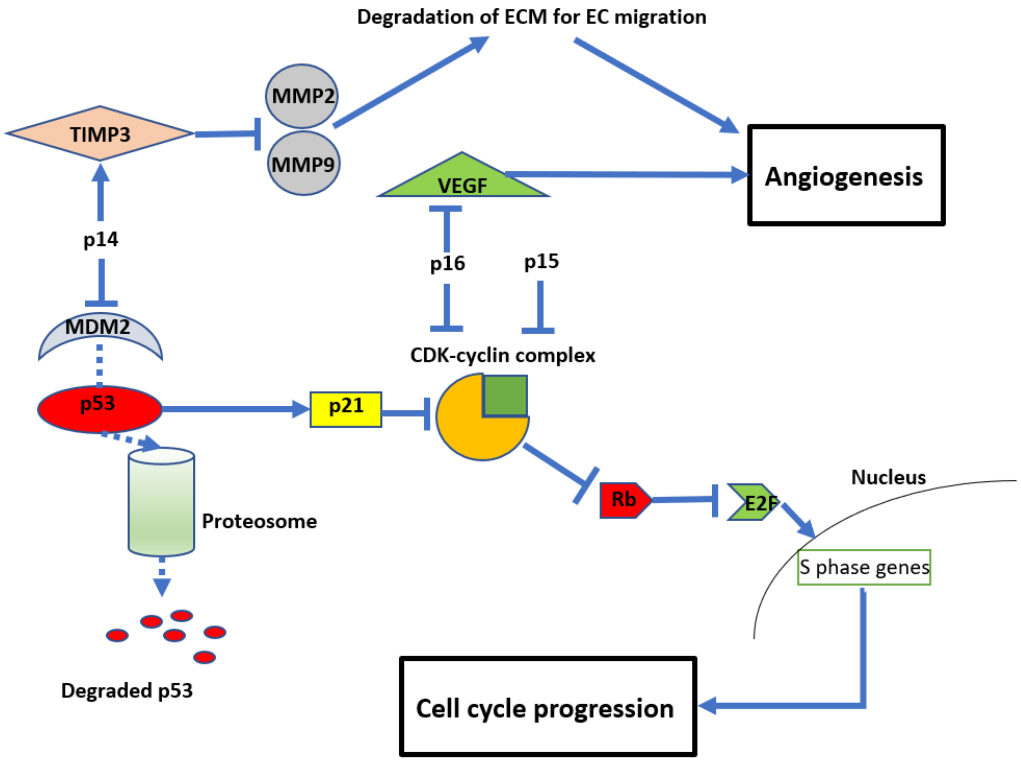

Figure 2. Diagram showing the anti-proliferative and anti-angiogenic effects of *CDKN2A/B* Mouse double minute 2 homolog (MDM2), tissue inhibitor of metalloproteinase 3 (TIMP3), matrix metalloproteinases (MMP).

In addition to their role in regulating cell growth, *CDKN2A/B* also impact angiogenesis (see **Figure 2**). For example, p14 (unrelated to its inhibition of MDM2) can also inhibit endothelial cell migration required for angiogenesis by stimulating the expression of tissue inhibitor of metalloproteinase 3 (TIMP3), which inhibits matrix metalloproteinases (MMP) 2 and 9 [31]. MMPs are required to degrade the extracellular matrix to allow endothelial cell migration and subsequent vessel formation [32][33].

3. Identification of CDKN2A/B Deletions

A variety of methods can be used to evaluate *CDKN2A/B* HD. These include single-nucleotide polymorphism (SNP) microarrays [34][35], next-generation sequencing (NGS) [36][37], DNA-based methylation studies [14][15][16][17][18], and fluorescent in situ hybridisation (FISH) [38][39]. It should be noted that the accuracy of this variety of methods depends on the specific assay types used, as the genomic/cytogenetic resolution of each method differs.

Although SNP arrays, NGS, and methylation arrays possess greater resolution for individual gene-level detection, many studies combine *CDKN2A* and *CDKN2B* in the assessment of HD [14][16][34][35][37]. The accuracy of these methods is determined by the degree and depth of coverage of the genes of interest. NGS methods used in the literature to date include targeted gene panels [36] and whole exome sequencing (WES) [37], whereas methylation arrays include a combination of the HumanMethylation450 (450k) and MethylationEPIC (850k) arrays (Illumina, San Diego, CA, USA) [14][15][16][17][18].

Fluorescence in situ hybridization (FISH) can be used to detect deletions and has been validated against methods utilising polymerase chain reaction (PCR) [40]. Thresholds of detection for FISH need to be around 20% to 30% tumour cells with HD [38][41]. A commonly used FISH probe in clinical diagnostic practise, the Vysis CDKN2A/CEP 9 FISH Probe Kit (Abbott Laboratories, North Chicago, IL, USA), is large and spans *CDKN2A, CDKN2B,* and *MTAP* genes [42].

Immunohistochemistry (IHC) has been used to identify *CDKN2A* HD in gliomas with mixed results. Given the close proximity of the *CDKN2A* and *MTAP* genes (see **Figure 2**), loss of *MTAP* immunoreactivity has been suggested as a surrogate for *CDKN2A* HD [43] and has been demonstrated in mesothelioma [44][45].

However, Satomi et al. did show that loss of p16 immunoreactivity correlated with clinical outcome in *IDH*-mutant astrocytomas [43]. While this is supported by other studies that demonstrated p16-negative tumours on IHC had a high negative predictive value for *CDKN2A* HD in adult and paediatric morphologic glioblastomas [46], other studies reported p16/*CDKN2A* discordance with the IHC method [39]. Sensitivity and specificity for p16 immunoreactivity in detecting *CDKN2A* HD have been reported as 78–94% and 70–82%, respectively [43].

4. *CDKN2A/B* Deletions in Clinical Studies

4.1. Initial Clinical Studies

Initial studies by Schmidt et al. [47] and Giani and Finocchiaro et al. [48] confirmed that CDKN2A HD was present in patients' tumours and not just in glioma cell lines but did not assess *CDKN2B*. Giani and Finocchiaro et al. demonstrated *CDKN2A* HD in over 30% of gliomas (not further defined) and CDKN2A HeD in 25% [48]. Moulton et al. analysed 27 glioblastomas (not further defined) and identified 9 with *CDKN2A* HD, 3 with a heterozygous deletion, and one with a point mutation [49].

4.2. Clinical Outcomes of CDKN2A/B Deletion in the Pre-Molecular Classification Era (Pre-2016 WHO CNS Tumour Classification)

4.2.1. Correlation with High- and Low-Grade Gliomas

Initial studies described the relationship between *CDKN2A/B* and biologic markers of tumour aggressiveness (tumour grade and Ki-67 index). Sonoda et al. suggested *CDKN2A/B* deletions may have a role in gliomagenesis and therefore more aggressive tumour biology. Using single-strand conformation polymorphism (SSCP) and quantitative polymerase chain reaction (qPCR), they showed an increased incidence of *CDKN2A/B* HD in high-grade gliomas (44%, n = 12/27) compared to low-grade gliomas (10%, n = 1/10) [50]. Building on this concept, Ono et al. (1996) used multiplex PCR to assess *CDKN2A/B* HD in 50 astrocytomas and found a positive correlation between the Ki-67 index and *CDKN2A* HD (5/20 grade 3 astrocytomas and 6/13 glioblastomas had *CDKN2A* HD). CDKN2A HD was not identified in 17 grade 2 astrocytomas [51].

4.2.2. Correlation with Survival

In 2006, Dehais et al. reported that *CDKN2A* HD was a negative prognostic factor in a heterogeneous group of gliomas that included anaplastic astrocytomas, oligoastrocytomas, and oligodendrogliomas. Although 1p/19q status was assessed, the authors did not identify which cases had *CDKN2A* HD and 1p/19q co-deletion [11]. However, other reports did not find an association between *CKDN2A* HD and clinical outcome [52][53]. This may reflect differences in methodology and/or patient selection for tumours classified by morphology alone. One of these studies (Rich et al.) used a DNA microarray to assess the prognostic impact of *CDKN2A* deletion in patients older than 50 years. Although *IDH* status was not reported in the study, this population was likely enriched for *IDH*-wildtype tumours, and it was later shown that *CDKN2A* deletions lack prognostic impact in these tumours [52].

4.3. Clinical Outcomes in the Post-Molecular Classification Era (Post-2016 WHO CNS Tumour Classification)

4.3.1. Incorporation of CDKN2A/B Status into the fifth Edition of the WHO Classification (2021)

In 2020, the Consortium to Inform Molecular and Practical Approaches to CNS Tumour Taxonomy (cIMPACT-NOW), upgrade 5, published recommendations for grading criteria and terminologies in *IDH*-mutant astrocytomas. After reviewing the literature on multiple potential prognostic biomarkers, including *CDKN2A/B* HD, other Rb pathway genes, *PIK3R1* and *PIK3CA* mutations, *PDGFRA* and *MYCN* amplification, reduced global DNA methylation, genomic instability (high copy number variants or somatic mutations), and mitotic activity and proliferation indices, they concluded that while "significant mitotic activity" should remain as a criterion for distinguishing grade 3 from grade 2 *IDH*-mutant astrocytomas, if *CDKN2A/B* HD, necrosis, or microvascular proliferation was present, a grade 4 designation was appropriate [7].

4.3.2. Literature That Supports CDKN2A/B Stratification

A prime example of supporting literature is Reis et al who were one of the earliest to report on the prognostic impact of *CDKN2A HD* in the setting of *IDH* mutations. They identified *CDKN2A* deletions as a prognostic marker specifically in *IDH*-mutant grade 2 and 3 gliomas. The authors analysed 270 gliomas and identified *CDKN2A* deletions via FISH in 57/108 grade 2 astrocytomas, 31/61 grade 3 astrocytomas, 23/96 oligodendrogliomas, and 19/49 oligoastrocytomas, inclusive of both homozygous and heterozygous *CDKN2A* deletions. The authors assessed tumours for 1p/19q deletion if they were not morphologic astrocytomas and assessed all tumours for *IDH1/2* mutations by genome sequencing. They reported worse overall survival in grade 2 and 3 gliomas after adjusting for age, sex, and *IDH* mutation (HR 1.6, 95% CI = 1.0–2.4, p = 0.03). This significance was maintained in the astrocytoma subgroup (HR 2.0, 95% CI 1.1–3.5, p = 0.02) but not for oligodendrogliomas or oligoastrocytomas (HR 0.7, 95% CI 0.2–2.0, p = 0.5 and HR 0.8, 95% CI 0.3–2.4, p = 0.7, respectively). Again, a portion of these morphologic oligodendrogliomas in this cohort would no longer be classified as such without the corresponding molecularly confirmed 1p19q co-deletion. Interestingly, the presence of deletions in the

IDH-mutant/*ATRX* expression loss astrocytoma group, without *TP53* mutation, was non-prognostic (p = 0.2) [54]. Furthermore, as *ATRX* loss and *TP53* mutations are strongly associated with *IDH*-mutant astrocytomas, it is unclear what this *ATRX*/*TP53* discordance represents in *IDH*-mutant gliomas. Interestingly, given the FISH probe used covers a broad genomic region at 9p21, *CDKN2B* status can be said to be assessed by proxy.

4.3.3. Literature That Counters CDKN2A/B Stratification

Not all studies supported the use of *CDKN2A/B* in *IDH*-mutant astrocytomas. One such example is Roy et al. who analysed the 9p region lost in malignancies by analysing two cohorts (the first group being 10,985 samples from 33 different cancer types and the second group being 540 low-grade gliomas from three databases) and reported that *CDKN2A* inactivation did not promote tumour aggressiveness. Even when accounting for *IDH* and 1p/19q status (*IDH*-mutant 1p/19q non-deleted astrocytoma), there was no survival impact of CKDN2A HD. While they did show that heterozygous loss was associated with poor OS, mRNA expression was not altered. It was therefore postulated that this survival impact was due to the loss of other 9p genes [55]. It is unclear why this report differs from the majority of other studies, but it highlights that not all studies support the role of *CDKN2A/B* HD as a prognostic marker in *IDH*-mutant astrocytomas.

5. Management of Tumours with CDKN2A/B Homozygous Deletions

There is no clear consensus on the treatment of *IDH*-mutant astrocytomas with *CDKN2A/B* HD, and reports related to their management are scarce. Reflecting this ambiguity, the current joint American Society of Clinical Oncology and Society of Neuro-Oncology guidelines recommend grade 4 astrocytomas be treated with concurrent temozolomide-radiotherapy with sequential temozolomide or radiotherapy alone with sequential temozolomide [56].

However, given the evidence that *CDKN2A/B* HD alters tumour biology (increased angiogenesis and cell growth), it cannot assume that these tumours will be as susceptible to temozolomide as their non-deleted counterparts. Unfortunately, the evidence for treatment specifically for *CDKN2A/B* HD astrocytomas is minimal. In 2000, Iwadate et al. investigated the relationship between *CDKN2A* deletion, p16 expression, and chemosensitivity to 30 different cytotoxic agents in vitro. They analysed 56 astrocytoma specimens (based on morphologic criteria, *IDH* status unknown) and found 17 specimens had p16 alterations (*CDKN2A* HD = 7, *CDKN2A* mutation = 5, p16 loss on IHC = 5). When looking at samples with p16 alterations, they found that deletions correlated with increased sensitivity to anti-metabolite agents but not to alkylating agents, antibiotics, topoisomerase inhibitors, or anti-microtubule agents [57].

6. Conclusions

CDKN2A/B HD have a direct oncogenic effect through loss of cell cycle inhibition and other parallel processes and are a molecular marker that influences grading and survival in *IDH*-mutant astrocytomas. Overall, the evidence supports the use of *CDKN2A/B* HD as a negative prognostic marker in *IDH*-mutant astrocytomas. However, there is a significant variation in certainty, methods used for deletion detection, and the quality of the presented literature. There are also inaccuracies resulting from misclassification of tumours in older studies based on the revised WHO classification. These limitations hamper conclusions regarding the certainty and depth of impact *CDKN2A/B* HD has on prognosis and management and how this impact is affected by other co-occurring molecular alterations. Therefore, the strongest evidence for *CDKN2A/B* HD in *IDH*-mutant astrocytomas must come from prospective reports with the current WHO 2021 classification.

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