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Review

Recent findings on molecular alterations in IDH1, TP53, and CASP9 in gliomagenesis

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ABSTRACT

Isocitrate dehydrogenase (IDH) mutations have been the focus of glioma-related neuroscience research since the discovery of the gene in 2008. IDH1 has been identified as a key enzyme in cellular metabolism, epigenetic regulation, redox regulation, and DNA repair and is thought to be one of the most important factors in gliomagenesis. IDH1 mutations cause neomorphic activity, resulting in an increase in 2-hydroxyglutarate production and a decrease in NADPH production. Emerging research has identified IDH1 mutations in the vast majority of low-grade gliomas and secondary glioblastomas, but these mutations are extremely uncommon in primary glioblastomas. Other genetic defects appear to play a significant role in glioma initiation and progression. In this study, we review recent findings on oncogenic alterations in IDH1, TP53, and CASP9 to identify any potential molecular correlations and interrelationships that lead to gliomagenesis. The roles and molecular interactions of these glioma-associated genes in gliomagenesis are elucidated. In addition, we highlight studies on stem cell modeling in glioma-associated genetic alterations that have been conducted over the past several decades.

Key words: brain tumour, glioma, genetic alterations, stem cell modelling

INTRODUCTION

Gliomas have been identified as the most prevalent primary cancer of the central nervous system (CNS) in humans. Initially, glioma classifications were based on specific histologic subtypes, with astrocytomas, ependymomas, and oligodendrogliomas being the most prevalent, followed by brainstem, optic nerve, and mixed gliomas. Glioblastoma is the most invasive, aggressive, and lethal type of glioma; this is classified as a high-grade glioma (HGG) (World Health Organization [WHO] grade IV) and has a poor prognosis[1]. The Cancer Genome Atlas (TCGA) identifies four subtypes of glioma based on their predominant genetic or epigenetic alterations in gene expression: 1) proneural, 2) neural, 3) mesenchymal, and 4) classical[2,3]. For instance, primary glioblastoma can be classified in any of the subtypes, whereas secondary glioblastoma is always classified as proneural. The WHO categorizes gliomas as low-grade gliomas (LGG; grades I and II) and HGGs (grades III and IV). LGGs are typically well-differentiated and slow-growing tumors, whereas HGGs are less differentiated, anaplastic, or diffusive tumors that infiltrate the brain parenchyma, thus making surgical resection challenging[4].

The discovery of isocitrate dehydrogenase isoform 1 (IDH1) mutations in gliomas has prompted extensive research into their direct and indirect roles in gliomagenesis[5]. IDH1 remains the benchmark for the subtype classification of gliomas. Its mutation is frequently associated with TP53 mutations in astrocytic tumors, but these tumors rarely exhibit co-deletion of chromosomes 1p and 19q, which is more prevalent in oligodendrogliomas[6–8]. TP53 mutations are uncommonly associated with oligodendrogliomas[7], while mutations in the capicua gene[6] are more common. **Figure 1** depicts a schematic summary of gliomagenesis based on the IDH1 mutation status.

Although IDH1 mutations are frequently observed in gliomas, they are uncommon in primary glioblastomas. Glioblastoma typically develops through a variety of pathways. Primary glioblastoma develops *de novo*, with or without lower-grade precursors, and acquires multiple complex genetic alterations in epidermal growth factor receptor (EGFR) and phosphatase and tensin homolog (PTEN)[6,7]. A mutation in the telomerase reverse transcriptase promoter has been linked with glioblastoma, in particular primary glioblastoma arising from astrocytic glioma, and grade II oligodendroglioma[9]. Secondary glioblastoma may also arise from grade II or III astrocytomas; this is frequently associated with loss of heterozygosity of chromosome 10q[10] but not chromosomal mutation +7/10 in the context of EGFR mutations typically found in astrocytic glioblastoma[11]. Oligoden-

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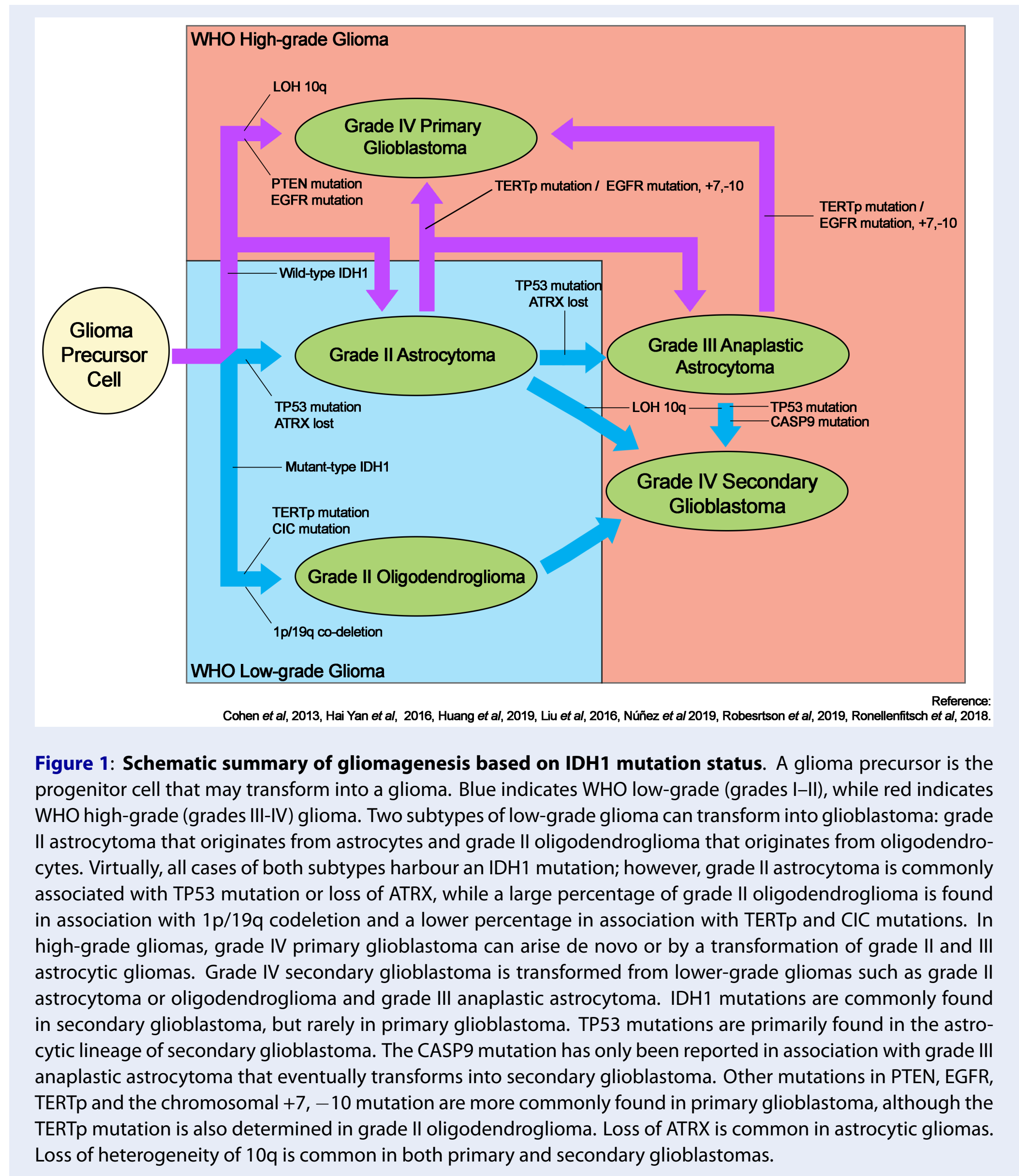

Figure 1: **Schematic summary of gliomagenesis based on IDH1 mutation status**. A glioma precursor is the progenitor cell that may transform into a glioma. Blue indicates WHO low-grade (grades I–II), while red indicates WHO high-grade (grades III-IV) glioma. Two subtypes of low-grade glioma can transform into glioblastoma: grade II astrocytoma that originates from astrocytes and grade II oligodendroglioma that originates from oligodendrocytes. Virtually, all cases of both subtypes harbour an IDH1 mutation; however, grade II astrocytoma is commonly associated with TP53 mutation or loss of ATRX, while a large percentage of grade II oligodendroglioma is found in association with 1p/19q codeletion and a lower percentage in association with TERTp and CIC mutations. In high-grade gliomas, grade IV primary glioblastoma can arise de novo or by a transformation of grade II and III astrocytic gliomas. Grade IV secondary glioblastoma is transformed from lower-grade gliomas such as grade II astrocytoma or oligodendroglioma and grade III anaplastic astrocytoma. IDH1 mutations are commonly found in secondary glioblastoma, but rarely in primary glioblastoma. TP53 mutations are primarily found in the astrocytic lineage of secondary glioblastoma. The CASP9 mutation has only been reported in association with grade III anaplastic astrocytoma that eventually transforms into secondary glioblastoma. Other mutations in PTEN, EGFR, TERTp and the chromosomal +7, −10 mutation are more commonly found in primary glioblastoma, although the TERTp mutation is also determined in grade II oligodendroglioma. Loss of ATRX is common in astrocytic gliomas. Loss of heterogeneity of 10q is common in both primary and secondary glioblastomas.

drogliomas may subsequently transform into anaplastic oligodendroglioma or secondary glioblastoma[12].

In the United States, glioma is the most prevalent cancer among children and young adults aged 20 to 39 years[13,14]. According to the National Cancer Institute's Surveillance, Epidemiology, and End Results Program, there were approximately 24,000 new cases of brain and other nervous system cancers in the United States in 2019, accounting for 1.4% of all new cancer cases[15]. In Malaysia, leukemia and lymphoma are surpassed by brain and CNS tumors as the third most prevalent cancer in children[16]. In accordance with Sustainable Development Goal 3 of the United Nations (good health and well-being), this global health issue warrants increased focus.

In 2016, the WHO revised its classification system for brain and spinal cord tumors by incorporating key genetic alterations into the classification[17,18]. Previously, the classification of primary and secondary glioblastomas was based solely on clinical and histopathological presentations; however, this method is subjective, resulting in misclassification. Histopathologically, high-grade tumors are indistinguishable[6]. Further, a number of alterations in genes, including IDH1[6,8], 1p/19q co-deletion[7], TP53[1], CASP9[19], PTEN[7], cyclin-dependent kinase inhibitor

2A[2], and EGFR[3], are associated with gliomas. This review focuses on IDH1, TP53, and CASP9 as potential drivers of gliomagenesis and seeks to identify potential correlations between these genes in gliomagenesis.

MOLECULAR ALTERATIONS IN IDH1, TP53, AND CASP9 IN GLIOMAGENESIS

IDH1 mutations were first discovered in LGGs but are also frequently found in HGGs[5–8,20]. Numerous studies have demonstrated a significant correlation between IDH1 and various histological tumor types, particularly secondary glioblastoma[6–8]. Over 80% – 90% of IDH1 mutations in gliomas are missense mutations that replace arginine (CGT) with histidine (CAT) at nucleotide 395 and codon 132 in exon 4 (IDH1R132H, c.395 G>A)[6–8,16,20–22]. Less common polymorphisms at codon 132 include IDH1^{R132C}, IDH1^{R132L}, IDH1^{R132S}, IDH1^{R132G}, IDH1^{R132V}, and IDH1^{R132P}[7–11,20–23]. Interestingly, IDH1^{R132C} has been associated with Maffucci syndrome[24], while IDH1^{R132L} has not been observed in immunohistochemistry[23]. Non-IDH1^{R132H} mutations were identified in DNA analyses but not in immunohistochemistry[25]. Non-IDH1^{R132H} mutations may be unique to secondary glioblastoma but evade detection in early LGG[23,25]. Recently, IDH1 mutations have been reported to acquire chemoresistance[26] and drug resistance[27]. Therefore, we strongly recommend incorporating molecular diagnostics into clinical settings, particularly for patients with glioma, to detect the IDH1 mutation status.

The p53 protein encoded by the TP53 gene plays a crucial role as the hub of the cellular regulatory network in regulating cell proliferation, senescence, apoptosis, genome integrity, and other regulatory functions[28–30]. Notably, TP53 gene mutations are one of the most prevalent biomarkers associated with gliomas, as they have been identified in virtually all cancers. Surprisingly, Yan *et al.* demonstrated that TP53 mutations were more prevalent in certain subtypes of gliomas such as diffuse astrocytomas, anaplastic astrocytomas, and secondary glioblastomas, which were also strongly associated with IDH1 mutations[8]; meanwhile, TP53 mutations were less commonly found in oligodendrogliomas or anaplastic oligodendrogliomas[28,29,31]. In addition, these tumors demonstrated 1p/19q co-deletion less frequently than did oligodendrogliomas, which yielded 1p/19q co-deletion almost universally, despite possibly harboring TP53 mutations[1]. The reported TP53 hotspot mutations in gliomas include S127P [nucleotide 379; serine (TCC) to proline (CCC)], R175H [nucleotide 524, arginine (CGC) to histidine (CAC)], G245S [nucleotide 733, glycine (GGC) to serine (AGC)], R248Q [nucleotide 743, arginine (CGG) to glutamine (CAG)], S260A [nucleotide 778, serine (TCC) to alanine (GCC)], R273H [nucleotide 818, arginine (CGT) to histidine (CAT)], and R273Y [nucleotides 817 and 818, arginine (CGT) to tyrosine (TAT)] (32). Kawasoe *et al.* reported that TP53 mutation was found in the early development of astrocytoma[32]. In contrast, TP53 mutations and 1p/19q co-deletion are nearly mutually exclusive.

Although germline mutations were rarely reported, somatic mutations account for many brain tumors. This is attributed to the fact that most gliomas are sporadic and have no known predisposing germline variants[30]. Since it was recently reported as a germline mutation found in patients with glioma, CASP9 gene mutation has garnered considerable research interest[19]. CASP9 mutations include R65X [nucleotide 193, arginine (CGA) to a stop codon (TGA)] and Q221R [nucleotide 662, glutamine (CAG) to arginine (CGG)][19,33]. The caspase-9 enzyme encoded by the CASP9 gene is a key biomolecule in the p53-dependent mitochondrial programmed cell death pathway. We hypothesized that CASP9 mutation and TP53-mediated gliomagenesis are closely related.

MOLECULAR ETIOLOGY OF GLIOMAS BASED ON IDH1, TP53, AND CASP9

IDH1 mutation causes profound changes at the cellular level, including alterations in cellular metabolism. IDH1 gain-of-function mutations reduce -ketoglutarate (-KG) to the oncometabolite (R)-enantiomer of 2-hydroxyglutarate (R-2-HG)[34]. Evaluation of clinical and cultured samples revealed elevated 2-HG levels in glioma cells harboring IDH1 mutation[7,35]. Eventually, the increased concentration of 2-HG reduces the production of NADPH and halts the oxidative decarboxylation of isocitrate[21]. IDH1^{R132H} mutation inactivates the ability to bind isocitrate catalytically and reduces the activity of the enzyme[35]. Competition between 2-HG and α-KG at catalytic sites further inhibits α-KG-dependent enzyme function, rendering cells susceptible to pharmacological glutaminolysis inhibition[36,37]. The α-KG-dependent enzymes include collagen prolyl-4-hydroxylase, prolyl hydroxylase, the ten–eleven translocation (TET) family of 5 methylcytosine hydroxylases, the Jumonji domain-containing family

of histone lysine demethylases, enzymes involved in nucleic acid metabolism, and other enzymes with still unknown functions[34]. The glutaminolysis pathway has been identified as being dependent on IDH mutations, and IDH1-mutant glioma has been hypothesized to rely on glutamate rather than on glutamine[38]. In fact, IDH1-mutant tissues display a significant decrease in glutamine levels but not in cell cultures[35]. Accordingly, IDH1^{R132H} mutation may be a driver mutation in gliomas likely through the production of 2-HG, which leads to the previously mentioned conditions. However, it remains unknown whether IDH1 mutation, 2-HG, or both promote oncogenic events.

IDH1 mutations may cause hypermethylation, gene-specific hypomethylation, and genome-wide hypomethylation, increasing G-CIMP (glioma CpG island methylator phenotype) production[39]. DNA methylation is essential for the regulation of gene activity and nuclear structure as well as the development and progression of cancer[40,41]. CIMP hypermethylation in the promoter regions of tumor-suppressor genes has been linked with numerous cancers[39]. The prevalence of hypermethylation is fourfold higher in glioma than in other cancers with IDH mutations[39]; this indicates that gliomas with IDH mutations have distinctive molecular characteristics compared with other cancers with IDH mutations, in which significantly elevated CIMP levels are rarely observed. Liu hypothesized that IDH1 mutation and the G-CIMP phenotype are unique to gliomas[22], while Kamiska claimed that G-CIMP is a prognostic factor[34], with low levels being associated with poor outcomes[30,39]. Similarly, loss of DNA methylation is associated with the progression of glioma[7]. In a neurosphere model, 2-HG is significantly associated with inhibition of lysine demethylases and the TET family, which results in hypermethylation of histone 3 and epigenetic reprogramming of the glioma transcriptome[39,40]. However, changes in histone methylation lead to the suppression of cell differentiation[34]. CIMP may not be the actual oncogenic transformation factor, at least in astrocytes, because IDH1 mutation introduction did not result in tumor formation[6]. Silencing epigenetic targets may reduce glioma malignancy and yield favorable clinical outcomes.

IDH1 protects cells against reactive oxygen species (ROS) by generating GSH. NADPH is also involved in lipid metabolism and contributes to cell defense against ROS during lipid oxidation[34]. Its mutation reduces the pool of GSH by decreasing the levels of -KG and NADPH, an essential cofactor for maintaining normal levels of GSH, resulting in increased susceptibility to ROS[38]. As a result of exposure to free radicals, cells become more susceptible to oxidative damage and eventually die[6]. Unexpectedly, *in vitro* studies have demonstrated an inverse relationship between GSH and ROS in cancer cells carrying IDH1 mutations. In neither the brain nor the hematopoietic cells of IDH1 knock-in animal models nor immortalized human astrocyte cell lines were the aforementioned conditions observed[38].

IDH1^{R132H} harboring glioma tumors and cultured glioma cells have recently been found to have reduced levels of β-oxidation and carnitine. Miyata was the first to discover that oxidation decreased only in IDH-mutant gliomas owing to the decrease in carnitine levels and that the difference in carnitine levels was significantly greater than that in 2-HG levels[35]. The changes in carnitine levels may explain why patients with IDH1 mutations have better prognosis than those with wild-type IDH1 mutations, as reduced β-oxidation activity inhibits tumor growth by depleting ATP for cancer cell division. Miyata suggested that carnitine is a better biomarker than 2-HG for detecting IDH mutations, given that patients with mutant-type IDH1 gliomas had significantly lower levels of carnitine than those with wild-type IDH1 gliomas[35]. Interestingly, the decrease in carnitine levels and β-oxidation activities varied considerably between clinical samples and cell lines[35]. The possibility of carnitine as a better biomarker and its relationship to the prognostic value in patients with glioma must be validated.

High levels of 2-HG have been known to inhibit prolyl hydroxylase domain (PHD) and hypoxia-induced factor 1 subunit alpha (HIF1-α), leading to angiogenesis that supports the development of glioma[34]. Thus, gliomas harboring mutant-type IDH1 express lower levels of HIF1-α than do those harboring wild-type IDH1. HIF1-α is also a transcription factor that aids in the adaptation to hypoxic conditions, thus impacting the hypoxia status, angiogenesis, metabolism, growth and differentiation, apoptosis and autophagy, and cell motility[34]. Moreover, Zhao suggested that PHD enzymes are the key drivers for HIF1-α degradation and hydroxylation[42]. These enzymes require α-KG and ferrous iron (Fe^{2+}) as a cofactor for the degradation of HIF1-α. Thus, IDH1 mutations that reduce the levels of α-KG may stimulate the cellular accumulation of HIF1-α[7]. As a result of decreased HIF1-α expression, vascular endothelial growth factor is overproduced, allowing angiogenesis and vasculogenesis critical for tumor growth and metastasis via the vasculature[6,7]. Increased HIF1-α levels and gene expression were also observed *in vitro* in U87MG cell lines, but the effect was abolished by exogenous

administration of an α-KG derivative[42]. In brief, IDH1 mutation induces an overproduction of 2-HG oncometabolite molecules, leading to the production of HIF1-α that supports the vascularization of tumors and allows the growth and metastasis of tumors.

IDH1 mutation can increase the sensitivity of gliomas to radiation[6]. However, increased survival in an animal model was only achieved with pharmacological treatment, not with radiation[40]. G-CIMP hypermethylation modulates glioma sensitivity to drugs and radiotherapy, which consequently enhances resistance to ionizing radiation[22]. This mutation induces genomic stability but reduces the efficacy of radiotherapy in animal models[40]; it further induces extensive DNA hypermethylation and reshaping of the methylome, mirroring G-CIMP-positive LGG in primary human astrocytes[43]. Accordingly, IDH1 mutation might decelerate the growth of glioma and increase DNA repair capacity, although it remains unclear whether it increases or decreases radiosensitivity. Patients with IDH1-mutated glioma generally exhibit a longer survival period than do patients with wild-type IDH1-mutated glioma, perhaps because of the effects of the mutation on G-CIMP. Almost all G-CIMP-positive tumors possess an IDH1 mutation, while no G-CIMP-negative tumors have been found to carry such mutation[43]. Thus, G-CIMP and IDH1 mutations are tightly associated. In the context of TP53 and ATRX inactivation, IDH1 mutation stimulates the ataxia–telangiectasia signaling pathway, consequently inducing DNA damage response and genomic stability owing to epigenetic reprogramming mechanisms involving chromatin modifications via histone lysine demethylation[33,40]. Although G-CIMP is a major determinant of gliomagenesis, its molecular basis remains poorly understood.

The p53 pathway is frequently deregulated in glioblastoma. TCGA reported that the p53 pathway is deregulated in >80% of gliomas[44] and up to 90% of glioblastoma cell lines[37]. The p53 pathway is disrupted in response to DNA damage and genotoxicity, oncogene activation, aberrant growth signaling, and hypoxia[1]. The particular mutations are typically missense mutations in the DNA-binding domain, abrogating transcription factor activity[1]. Under normal conditions, p53 ubiquitination is mediated by ubiquitin ligase in a negative feedback loop to regulate p53 protein activity. However, this is disrupted by TP53 missense mutations. In contrast to wild-type TP53, mutant-type TP53 results in loss of wild-type function and gain-of-function, causing the accumulation of p53 proteins, which then triggers enhanced proliferation, invasion, reduced chemosensitivity, carcinogenic metabolism, disturbed tissue architecture, and tumor initiation and progression[1,28]. A shorter survival was observed in patients with mutant-type TP53 and IDH1, while the prognosis of patients with wild-type IDH1 was not affected[1,6]. Although TP53 mutation is found in almost all cancer types, TP53 mutation status allows better understanding of the tumor biology of gliomas, especially in the context of co-occurrence with other gene mutations.

CASP9 R65X mutation causes loss of the catalytic domain of caspase-9. This mutation is predicted to have functional consequences in the p53 signaling pathway. In response to DNA damage, p53 promotes pro-apoptotic biomolecules and inhibits anti-apoptotic biomolecules, activating caspase-9[19]. The active form of caspase-9 triggers a cascade of effector caspases, resulting in cell death. However, CASP9 mutation blocks the p53 programmed cell death cascade, allowing the survival of DNA-damaged cells. Apoptosis is defined as a physiological process of programmed cell death; thus, defects in this mechanism can lead to abnormal cell growth and proliferation. A summary of the roles of IDH1, TP53, and CASP9 gene mutations based on 2-HG production in gliomagenesis is shown in **Figure 2**. Additional studies are needed to examine whether IDH1, TP53, and CASP9 mutations are involved in tumor progression or result from a damaged DNA repair mechanism in tumors.

STEM CELL MODELING IN GLIOMA-ASSOCIATED GENETIC ALTERATIONS

There are increasing efforts to manipulate the current technology of genetically modified or orthotopic induction animal models that develop malignant brain tumors. Owing to animal ethics, different assays or paradigms to enrich brain tumor biology, including that of cancer stem cells, have been used in brain tumor research[45]. One frequently used system involves placing primary human GBM cells in suspension with serum-free defined growth factors[46]. This approach has been used to study *in vitro* growth properties, chemosensitivity and chemoresistance, epigenetic characteristics, and gliomagenesis potential[46,47]. Another system is an immunological approach in which antibody-mediated selection for putative glioma cell-specific epitopes is applied[47]. Examples of the well-known epitopes of glioma cells are CD133, CD44, CD15, and SSEAs[47]. Brain stem cells are enriched in populations by the ability to efflux the Hoechst 33342 dye. Next-generation sequencing has played an important role in deciphering the genomic landscape of gliomas, therefore leading

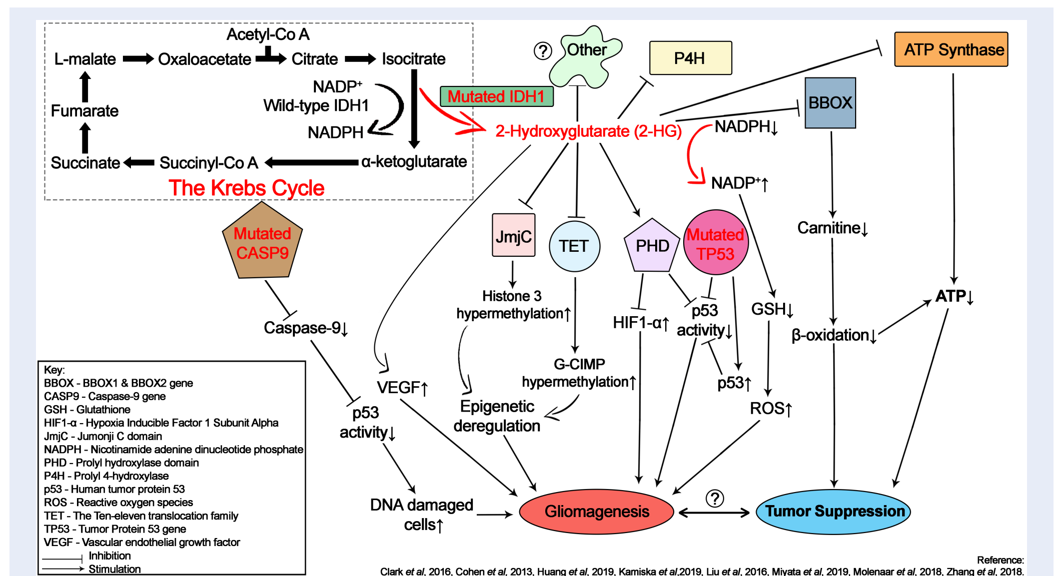

Figure 2: **The role of IDH1, TP53, and CASP9 gene mutations based on 2-HG production in gliomagenesis**. The dotted square represents the Krebs cycle. In normal conditions, IDH1 catalyzes isocitrate into α-KG with $NADP^+$ cofactor. However, IDH1 mutations often lead to catalysis of isocitrate into 2-HG, with NADPH as the cofactor. 2-HG is associated with the overproduction of VEGF, which promotes angiogenesis and vasculogenesis, thus driving glioma progression. 2-HG inhibits the JmjC domain and TET family proteins, resulting in histone 3 hypermethylation and G-CIMP hypermethylation, respectively, leading to epigenetic deregulation and gliomagenesis. 2-HG reduces PHD enzyme activity, leading to HIF1-α accumulation and inhibiting the p53 signalling pathway, which may cause glioma initiation and progression. In addition, 2-HG inhibits P4H and other unknown proteins; it is also reported to inhibit ATPase and reduce ATP production, which eventually inhibits cell growth, including tumour cells, thereby contributing to tumour suppression. A very recent study showed that 2-HG inhibits γ-butyrobetaine dioxygenase activity. Mutation in the TP53 gene has a direct causal effect on the p53 signalling pathway, which has been deemed vital in regulating programmed cell death, thus leading to no removal of mutated cells such as tumour cells, allowing the cells to survive. TP53 mutation also stimulates the production of mutated p53 proteins. CASP9 mutation was reported to reduce its protein production and interrupt the p53 signalling pathway; thus, the tumour cells escape apoptosis. Despite the clear association between IDH1 mutation, gliomagenesis, and tumour suppression, the link between these extremes remains controversial. We believe there might be as-yet-undiscovered novel proteins or enzymes which may mediate gliomagenesis or tumour suppression initiated by IDH1, TP53, or CASP9 gene mutations.

to the era of molecular biology- and stem cell-based approaches for the study of genetic alterations in gliomas. More recently, induced pluripotent stem cell (iPSC) technology, 3D bioprinting, and 3D culture technique have been introduced to model gliomas. Herein, we describe stem cell modeling in glioma-associated genetic alterations as they have been thoroughly investigated.

The cancer stem cell theory postulates that tumors are sustainable owing to their specific features, including self-renewal ability. Neural stem cells are normally non-dividing populations but can be induced to proliferate under conditions of stress [48]. Llagun and Parada suggested that glioma stem cells originate from a neural stem cell; therefore, glioma and neural stem cells share common phenotypic markers [46,48]. Glioma stem cells are distinguishable based on their genetic alterations. The most frequent genetic alterations found in gliomas are alterations in the genes that encode proteins implicated in signaling cascades or cell cycle control, as previously described.

Fantin and colleagues experimented on U87MG and LN-18 human glioblastoma stem cells transfected with Myc-tagged wild-type IDH1 and mutant-type IDH1^{R132H} mutations to profile the changes in 2-HG levels between glioma stem cell models [49]. They found that IDH1^{R132H}-expressing stem cells had a significantly higher level of 2-HG than wild-type IDH1-expressing stem cells [49]. The 2-HG level is elevated in mutant-type IDH1 tumor samples. The stem cell model could be mimicking mutant-type IDH1-specific glioma stem cells. The use of glioma stem cell modeling for investigating glioma-associated genetic alterations might be reliable and be an alter-

native method for animal models, supporting the 3R concept (Replacement, Reduction, Refinement) in brain tumor research. This notion was further supported by the findings by Shi *et al.* who used two different human glioblastoma cell lines (U87MG and U251MG) to demonstrate IDH1 gene alterations by site-directed mutagenesis and lentivirus transfection[50]. They found that the IDH1 mutation stem cell model showed increased chemosensitivity, decreased GSH and NADPH levels, and increased ROS production, similar to the changes found in patient tumor samples in other studies[50].

Olafson *et al.* analyzed mutated genes from a glioma patient-derived cell line model. Two low-passage patient-derived primary cell lines were established in the laboratory and then analyzed via pyrosequencing, *in situ* hybridization, immunohistochemistry, and next-generation sequencing to validate gene mutations in the stem cell model[28]. They found *PTEN*, *EGFR*, *MAP3KI*, *NTRKI* and *TP53* gene mutations in both cell line models in next-generation sequencing, although they were able to detect mutant-type *TP53* gene in only one of the stem cells[28]. The mutational data revealed molecular profiles similar to tumors.

More advancements have been achieved by integrating iPSC technology into the study of glioma-initiating cells[51] and modeling of LGGs[51]. Further, 3D bioprinting as a scaffold for cell culture was recently used to study the invasion ability of glioma[52]. The discovery of cerebral organoids[53] has opened up a new research niche in the investigation of gliomas. Several studies demonstrated that 3D culture of cerebral organoids provides a superior understanding of glioma at another level[54–56].

Stem cell modeling in glioma-associated genetic alterations is an essential alternative method from animal models. An increasing number of studies use stem cell models in brain tumor biology. Although the roles of several genes in glioma stem cell models remain uncertain, stem cell modeling for gliomas can reveal valuable information for brain research.

CONCLUSIONS

The metabolic and biological consequences of genetic mutations can improve the understanding of the development and clinical behavior of different molecular subtypes of glioma. However, the ability to target gliomas with IDH mutations remains limited. Thus, an in-depth understanding of the fundamental biology of gliomas is warranted to improve the knowledge of tumor biology and will be of great clinical and prognostic importance for neuroscience. Discoveries of targets and therapeutic strategies may soon emerge.

ABBREVIATIONS

2-HG: 2-hydroxyglutarate, **3R's**: replacement, reduction and refinement, **ATP**: adenosine triphosphate, **CAG**: glutamine, **CASP9**: Caspase-9, **CAT, CAC**: histidine, **CCC**: proline, **CD133, CD44, CD15**: cell of differentiation 133, 44, 15; **CGT, CGC, CGG**: arginine, **CIMP**: CpG island methylator phenotype, **DNA**: deoxyribonucleic acid, **EGFR**: epidermal growth factor receptor, **GBM**: glioblastoma, **GCC**: alanine, **G-CIMP**: glioma CpG island methylator phenotype, **GGC**: glycine, **GSH**: glutathione, **HGG**: high grade glioma, **HIF1-** α: hypoxia-induced factor 1 subunit alpha, **IDH**: Isocitrate dehydrogenease, **IPSC**: induced pluripotent stem cell, **JmjC**: Jumonji domain-containing family, **LGG**: low grade glioma, **MAP3KI**: mitogen-activated protein kinase 1, **NADPH**: Nicotinamide adenine dinucleotide phosphate, **NTRKI**: neurotropic tyrosine receptor kinase 1, **PHD**: prolyl hydroxylase domain, **PTEN**: phosphatase and tensin homolog deleted on chromosome 10, **R-2-HG**: R-enantiomer of 2-hydroxyglutarate, **ROS**: reactive oxygen species, **SSEAs**: stage-specific embryonic antigens, **TAT**: tyrosine, **TCC, AGC**: serine, **TCGA**: The Cancer Genome Atlas, **TET**: ten-eleven translocation, **TGA**: stop codon, **TP53**: tumor protein 53, **VEGF**: vascular endothelial growth factor, WHO: world health organization, α**-KG**: alpha-ketoglutarate

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Conceptualization, JYP, ZI, AAMY, and AP Writing—original draft preparation, JYP; writing—review and editing, ZI, AAMY, and AP; supervision, AP.; funding acquisition, ZI, AAMY, and AP. All authors have read and agreed to the published version of the manuscript.

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CONSENT FOR PUBLICATION

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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