Emerging Pathogenic and Prognostic Significance of Paired Box 3 (PAX3) Protein in Adult Gliomas

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Abstract

Gliomas present the most common type of brain tumors in adults, characterized by high morbidity and mortality. In search of potential molecular targets, members of paired box (PAX) family have been found expressed in neural crest cells, regulating their proliferation, apoptosis, migration and differentiation. Recently, PAX3 overexpression has been implicated in glioma tumorigenesis by enhancing proliferation, increasing invasiveness and inducing resistance to apoptosis of glioma cells, while maintaining brain glioma stem cells (BGSCs) stemness. Although the oncogenic potential of PAX3 in gliomas is still under investigation, experimental evidence suggests that PAX3 function is mainly mediated through the canonical and non-canonical Wnt signaling pathway as well as through its interaction with GFAP and p53 proteins. In addition, PAX3 may contribute to the chemoresistance of glioma cells and modulates the effectiveness of novel experimental therapies. Further evidence indicates that PAX3 may represent a novel diagnostic and prognostic biomarker for gliomas, facilitating personalized treatment. This review addresses the emerging role of PAX3 in glioma diagnosis, prognosis and treatment, aiming to shed more light on the underlying molecular mechanisms that could lead to more effective treatment approaches.

Translational Oncology (2019) 12, 1357–1363

Introduction

Gliomas are the most common types of primary central nervous system (CNS) tumors in adults, accounting for approximately the 80% of all malignant types [1]. Their peak incidence is observed in the fifth and sixth decades of life and they occur more frequently in men [2]. Despite extensive research efforts towards the elucidation of the molecular pathways involved in glioma development and progression, these tumors are still associated with high morbidity and mortality and only 13% of patients reach an overall 5-year survival [3]. Even after optimal treatment that involves surgical resection followed by radiation and chemotherapy, gliomas are characterized by high recurrence rates. The excessive cell proliferation, invasive growth and resistance to apoptosis along with the blood brain barrier limitation for effective delivery of chemotherapeutic agents, contribute to therapeutic failure [4].

Traditional classification of gliomas into oligodendroglial, astrocytic, oligoastrocytic and ependymal tumors was originally based on microscopic histological characteristics as defined by the 2007 World Health Organization (WHO) classification of CNS tumors [5].

However, in the past two decades, the identification of distinct genetic and epigenetic alterations in gliomas has revealed significant biomarkers with prognostic potential within each morphologically defined glioma subtype that can lead to effective personalized treatment. The updated 2016 WHO Classification of CNS tumors incorporates for the first time molecular markers in addition to histologic characteristics, representing a revolutionary integrated approach [6]. Specifically, mutations in isocitrate dehydrogenase 1 *(IDHI)* and 2 *(IDHII)* genes are detected only in infiltrating

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astrocytomas and oligodendrogliomas, but not in ependymomas or circumscribed astrocytomas. *IDH* wild-type glioblastomas (GBMs), accounting for more than 90% of all GBMs, usually develop *de novo* in older patients, without a pre-existing lesion, whereas *IDH* mutant GBMs typically occur in younger adults with a pre-existing anaplastic or diffuse astrocytoma [6]. Importantly, for many of the glioma biomarkers identified, novel treatment strategies are currently under investigation, including monoclonal antibodies targeting *IDH1* mutations [7]. Therefore, given the newly established molecularly-oriented approach towards the diagnosis and management of gliomas, the discovery of novel molecular biomarkers synthetizing the molecular profile of distinct glioma subtypes is of paramount importance, paving the way for improved diagnosis, prognosis and effective personalized treatment.

The paired box *(PAX)* gene family consists of nine members *(PAX1-PAX9)* which are highly conserved. These encode transcription factors that are significantly implicated in the differentiation of various cell lineages during embryonic development as well as in the maintenance of multiple stem cell niches in adults [8].

PAX genes have been shown to act as either tumor promoters or suppressors and are implicated in the pathogenesis of several human cancers, including brain tumors. PAX2 has been found expressed in medulloblastoma cells and associated with a more aggressive phenotype [9] while PAX5 expression levels were correlated with increasing malignancy of astrocytomas [10]. On the contrary, PAX6 inhibited glioma cell proliferation and invasiveness and affected chemoresistance to temozolamide [11,12]. It further downregulated the expression of vascular endothelial growth factor A (VEGFA), thus suppressing angiogenesis [13]. Finally, PAX8 was shown to increase cell survival [14] and regulate telomerase reverse transcriptase in gliomas [15]. However, the clinical and prognostic significance of these PAX proteins in gliomas has not been established and is still under investigation.

Among the other family members, PAX3 is the most important, playing a prominent role in gliomagenesis. PAX3 is mainly expressed in neural crest, neural tube and embryonic limb muscle, where it regulates the differentiation, proliferation, migration, adhesion and apoptosis of neural crest and muscle precursor cells, respectively [16]. It has been recently demonstrated that persistent PAX3 expression in neuronal crest could result in cleft palate, sphenoid bone malformation and perinatal lethality in mice [17]. Notably, a growing body of evidence has shown that PAX3 may play diverse roles in oncogenesis since it is upregulated in various human malignancies, including melanoma, Ewing sarcoma, small cell lung and breast cancer, whereas it mainly acts as a tumor suppressor in thyroid cancer [18]. Mechanistically, PAX3 induces tumorigenesis in rhabdomyosarcoma by modulating PTEN, c-MET and activator protein 2 (AP-2) signaling pathways. In melanoma, PAX3 contributes to oncogenesis by regulating the expression of various genes, including the cell surface glycoprotein *MUC18 (CD146)*, *Transforming growth factor beta* (TGF-β) and Tissue Inhibitor of Metalloproteinase 3 *(TIMP3)* [19]. Interestingly, a growing body of data has revealed that PAX3 is highly implicated in cell proliferation, apoptosis and invasiveness of glioma cells, contributing to glioma tumorigenesis and progression. Additionally, it affects the resistance to chemotherapy and other treatments against gliomas that are currently under investigation. Accumulating data suggest that it could be an important diagnostic and prognostic marker for glioma tumors and may represent a promising therapeutic target.

In this review, we discuss recent experimental evidence on the emerging role of PAX3 in gliomagenesis with potential effects on diagnosis, prognosis and treatment.

The Role of PAX3 in Cell Proliferation and Apoptosis in Gliomas

Similar to other malignant tumors, gliomas are characterized by a highly proliferative phenotype mainly attributed to deregulation of cell-cycle control along with inhibition of apoptotic pathways. In this regard, PAX3 expression levels have been associated with cell proliferation in neuronal cells [20] and myoblasts [21] *in vitro*. Furthermore, PAX3 promotes tumorigenesis by enhancing cell proliferation in the case of alveolar rhabdomyosarcoma [22], while it acts as a tumor suppressor in the case of thyroid cancer [18].

Until recently, the specific effects of PAX3 on glioma tumorigenesis and the underlying molecular pathways remained largely unknown. *In vitro* studies have unraveled the significant implication of PAX3 in glioma cell proliferation and apoptosis. Human glioma U87 cells transfected with PAX3-small interfering RNA (siPAX3) that blocks PAX3 expression, as well as SHG44 cells transfected with the Flag-PAX3 plasmid that promotes PAX3 expression, revealed that PAX3 may enhance proliferation and suppress apoptosis in glioma cells [23]. Furthermore, another study that used a Flag-PAX3-over-expressing vector demonstrated that PAX3 can promote cell proliferation and cell cycle progression, while it can inhibit apoptosis in U87 glioma cell lines [24]. In agreement with the above, microRNA-485-5p (miR-485-5p), which directly binds to the 3'-UTR of PAX3 and inhibits its expression, can attenuate cell proliferation and induce cell cycle G1 arrest in U251 and U87 glioma cell lines [25]. Moreover, functional annotation of mRNA profiling and transcriptome analysis from glioblastoma specimens from the Chinese Glioma Genome Atlas (CGGA) and The Cancer Genome Atlas (TCGA) databases indicated that patients with higher PAX3 expression levels exhibited an increased expression of genes associated with cell proliferation [26]. These findings from these larger and low-biased datasets confirm the pivotal role of PAX3 in glioma cell proliferation. In the same study, the use of siPAX3 was shown to inhibit proliferation in U87 glioblastoma cells.

The underlying molecular mechanisms of the proliferative and anti-apoptotic properties of PAX3 in gliomas have not been clarified yet but they seem to involve increased B-cell lymphoma 2 (Bcl-2) expression levels, without altering Bcl-2 Associated X (Bax) expression or *AKT* Serine/Threonine Kinase 1 (AKT) phosphorylation [23], and reduction of c-Jun N-terminal kinase (JNK) phosphorylation [23,25]. Furthermore, it has been shown that the effects of PAX3 on proliferation and apoptosis in gliomas were mediated by the direct interaction of PAX3 with β-catenin, as evaluated by co-immunoprecipitation, as well as the potential subsequent regulation of Wnt signaling pathway [24]. In particular, PAX3 overexpression altered the expression of β-catenin, vascular endothelial growth factor (VEGF), Myc, matrix metalloproteinase 7 (MMP7), and CyclinD1, which are strongly associated with Wnt signaling pathway. In addition, a very recent study demonstrated that miR-362-3p suppressed glioma cell proliferation by targeting PAX3 and inhibiting β-catenin-dependent Wnt pathway [27].

Notably, abnormal activation of Wnt pathway plays a key pathogenic role in gliomas [28], since it is strongly associated with resistance to radio- and chemo-therapy, poor prognosis, cell invasiveness and maintenance of the stem cell properties of the

glioblastoma stem cells (GSCs) [29]. Mechanistically, the activation of the canonical Wnt pathway firstly involves the formation of a trimer complex, composed of Wnt proteins, receptors of the frizzled (FZD) and low-density lipoprotein-receptor-related protein (LRP) on the cellular membrane. This complex activates the protein disheveled (DVL), leading to the inhibition of glycogen synthase kinase (GSK-3β). Consequently, phosphorylated β-catenin escapes from degradation and translocates into the nucleus, where it affects the transcription of target genes implicated in cell proliferation, apoptosis and migration, including CyclinD1, VEGF and MMP7 [30]. Regarding the β-catenin-independent non-canonical Wnt signaling cascade Wnt proteins bind to FZD receptors and modulate the activity of protein kinase C (PKC) and JNK, leading to cytoskeletal alterations and the regulation of genes associated with glioma cell proliferation and apoptosis [31,32].

Several lines of evidence have reported the direct and indirect role of PAX3 in Wnt signaling cascade. PAX3 is required for neural crest formation in vertebrate embryos in a Wnt-dependent manner [33]. Also, PAX3 inductive signals from posterior non-axial mesoderm are Wnt-dependent while PAX3 expression in the neural plate depends on Wnt pathway [34]. Induction of PAX3 expression during neural crest development at the neural plate border is indirectly mediated by Wnt pathway, involving Caudal-related Homeobox (Cdx) proteins as intermediates [35]. Furthermore, PAX3 transcriptional activity is necessary for the induction of *MyoD* expression in explants of pre-somitic mesoderm (PSM) during myogenesis, *via* the activation of PKC-dependent non-canonical Wnt pathway [36].

This evidence suggests that PAX3 may regulate cell proliferation and apoptosis in gliomas at least partially *via* canonical and non-canonical Wnt signaling cascade, although the underlying molecular mechanisms need further elucidation.

Additional *in vivo* experiments have also confirmed the oncogenic properties of PAX3 in gliomas [23,25]. In particular, the subcutaneous inoculation of siPAX3-transfected U-87MG glioblastoma cells displaying decreased PAX3 expression in nude mice led to reduction of tumor size [23]. Accordingly, in subcutaneous glioma models in nude mice using flag-PAX3-transfected SHG44 cells, PAX3 overexpression promoted gliomagenesis [23]. Furthermore, increased expression of miR-485-5p that suppresses PAX3 expression can attenuate glioma growth *in vivo*, since the xenograft tumors from miR-485-5p-treated mice demonstrated a dramatic decrease in tumor growth, compared to controls [25]. Hence, *in vitro* and *in vivo* evidence strongly supports the significant contribution of PAX3 to tumorigenesis of gliomas and PAX3-targeted treatment strategies could represent a novel therapeutic approach.

The Role of PAX3 in Cell Invasion and Migration in Gliomas

Besides their uncontrolled proliferation and resistance to apoptosis, glioma cells are characterized by prominent invasiveness into the surrounding brain parenchyma, contributing to rapid infiltrative growth and high recurrence rates after surgical resection. Given the known key role of PAX3 in the migration of neural crest cells during development [37], it has been proposed that it could enhance the invasive potential of glioma cells. Indeed, a recent study demonstrated that siPAX3-transfected U87 glioma cells displayed a decreased invasive capacity [23]. This was further confirmed by the use of Flag-PAX3-transfected SHG44 glioma cells, which resulted in a significant increase of the number of invading cells, as evaluated by

the matrigel invasion assay [23]. Moreover, another study indicated that knockdown of PAX3 *via* siPAX3 in U87 glioblastoma cells significantly suppressed cell migration, as detected by the Transwell migrate test [26].

Regarding the molecular mechanisms involved, it has been shown that PAX3 may promote glioma cell invasiveness *in vitro* by reducing E-cadherin and increasing MMP-2 expression. In this context, previous studies have reported that E-cadherin expression (corresponding to epithelial phenotype) is rarely observed in glioma tumors, whereas a mesenchymal phenotype reflected by N-cadherin expression was identified in most glioma specimens from Grade I to Grade IV [38]. Furthermore, MMP-2 expression, an enzyme that degrades extracellular matrix and contributes to invasiveness of many cancers, has been shown to play a substantial role in glioma progression and it has been recently associated with high aggressiveness of astrocytomas and shorter survival of glioblastoma patients [39]. Furthermore, β-catenin-dependent Wnt pathway may also contribute to PAX3-mediated glioma cell migration, since miR-362-3p that directly targets PAX3, inhibited migration and epithelial-mesenchymal transition of glioma cells *in vitro* [27]. Consequently, PAX3 represents a critical mediator of the invasiveness of glioma cells, probably contributing to its highly infiltrating phenotype and rapid progression (Figure 1).

The Role of PAX3 in Maintaining BGSCs Stemness in Gliomas

Gliomas are suggested to evolve from brain glioma stem cells (BGSCs) which play a critical role in their occurrence, progression and resistance to conventional treatment, including surgery, chemotherapy and radiation. Therefore, elucidating specific molecular pathways implicated in the stemness maintenance of BGSCs are of paramount importance, since the development of BGSC-targeted therapies is proposed to represent a promising treatment strategy in the future.

In this context, recent evidence supports the critical contribution of PAX3 to the tumorigenic capacity and differentiation of BGSCs in gliomas [40,41]. Interestingly, it has been previously shown that PAX3 is able to bind to the promoter region of the glial fibrillary acidic protein (*GFAP)* gene, leading to the suppression of the expression of GFAP, an astrocyte maturation marker that is majorly implicated in the differentiation of astrocytes from neuronal stem cells (NSCs) [42]. GFAP expression has been also found to be downregulated in gliomas and is associated with tumor grades [43]. In this regard, PAX3 expression was significantly higher in BGSCs compared to U87MG glioma cells and normal astrocyte lines [40]. More importantly, PAX3 could bind to the promoter region of the *GFAP* gene, leading to repression of *GFAP* transcription. Furthermore, *PAX3* knockdown *via* the use of siPAX3 suppressed proliferation, promoted apoptosis and reduced the invasive potential of BGSCs in this study. On the contrary, PAX3 overexpression by plasmid vectors enhanced proliferation, inhibited apoptosis and increased the invasiveness of BGSCs. In addition, PAX3 could act as a transcriptional repressor during serum-induced differentiation of BGSCs [40]. In agreement with the above evidence, it has been demonstrated that PAX3 overexpression may maintain the high malignancy of gliomas by negatively modulating GFAP expression in glioma cell lines [23]. Taken together, PAX3 may affect the malignant potential of gliomas, at least partially by inhibiting *GFAP* transcription, resulting in the modulation of BGSCs differentiation, proliferation and invasiveness.
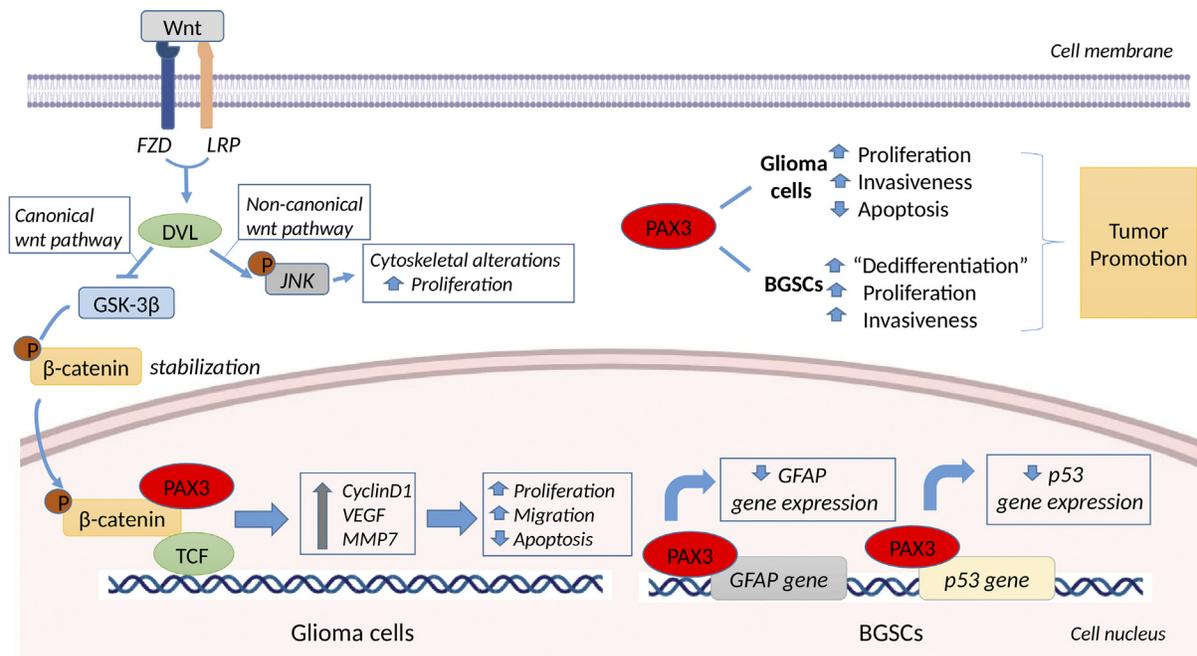
Figure 1. Proposed molecular mechanisms of PAX3 in glioma pathogenesis and progression. In glioma cells, the activation of the canonical Wnt pathway involves the binding of Wnt to the receptors of the frizzled (FZD) and to the low density lipoprotein-receptor-related protein (LRP) on the cellular membrane, resulting in a trimer complex formation that enters the cytoplasm. This complex promotes the phosphorylation and subsequent activation of the protein disheveled (DVL), leading to the inhibition of glycogen synthase kinase (GSK-3β). As a result, phosphorylated β-catenin escapes from degradation and translocates into the nucleus, where it can bind to T-cell transcription factor (TCF), leading to the abnormal transcription of target genes implicated in cell proliferation, apoptosis and migration, including CyclinD1, vascular endothelial growth factor (VEGF) and matrix metalloproteinase 7 (MMP7).Regarding the non-canonical Wnt signaling pathway, Wnt binds to FZD and activates the small GTPases, Rho and Rac, as well as JNK, resulting in cytoskeleton alterations and activation of transcription factors belonging to activator protein-1 (AP-1) family, which further regulate the expression of cell proliferation and apoptosis-associated genes. PAX3 has been shown to directly interact with β-catenin, and is also associated with reduction of JNK phosphorylation, as well as altered expression of VEGF, Myc, MMP7 and CyclinD1, implying its implication in both canonical and non-canonical Wnt pathway. In respect of brain glioma stem cells (BGSCs), PAX3 promotes their "dedifferentiation", proliferation and invasivness by suppressing *GFAP* and *p53* gene expression.

Furthermore, another study found that PAX3 is an essential regulator of self-renewal, differentiation, migration and oncogenic properties of BGSCs in gliomas both *in vitro* and *in vivo*, via a p53-dependent manner [41]. In particular, PAX3 enhanced the viability and migration of BGSCs, by transcriptionally suppressing p53 expression, probably through binding to the promoter region of the *p53* gene. Notably, PAX3 overexpression was associated with more *p53* mutations in glioma samples. Moreover, PAX3 played a substantial role in the hypoxia-promoted BGSCs persistence, since hypoxia-induced HIF-1α upregulation resulted in PAX3 overexpression, which further inhibited p53 expression, leading to the enhancement of "dedifferentiation" of the remaining differentiated BGSCs. Importantly, *p53* inactivation is a critical process during the development and progression of gliomas [44] and *p53* mutations have been already related to gliomagenesis. Hence, PAX3/GFAP and PAX3/p53 axes seem to be critically involved in glioma development and recurrence, and pharmaceutical targeting of these pathways could be of great value towards their treatment (Figure 1).

PAX3 as a Potential Diagnostic and Prognostic Biomarker in Gliomas

It is well established that the prominent molecular heterogeneity of gliomas largely contributes to their high recurrence rates even after optimal aggressive treatment. However, recent advances in next-gen-eration sequencing methods have shed light on the characterization of specific molecular signatures of gliomas, paving the way for the development of potential biomarkers [45]. Novel diagnostic biomarkers may improve the current glioma subclassification and prognostic biomarkers may effectively monitor disease progression and survival, while predictive biomarkers may enable a more patient-specific treatment approach.

Interestingly, PAX3 overexpression is associated with higher degrees of malignancy and poorer prognosis in various tumor types, including breast cancer and rhabdomyosarcoma [46,47]. In this context, a recent study that investigated PAX3 expression in brain specimens from 57 glioma patients and 10 controls *via* RT-PCR, Western blot and immunohistochemical staining demonstrated that PAX3 mRNA and protein levels were increased in high-grade (Grade III and IV) glioma tissues in comparison to low-grade (Grade I and II) and normal brain specimens [48]. More importantly, patients with increased PAX3 expression displayed worse overall survival, independent of age, gender, Karnofsky Performance Status (KPS) score, as well as tumor size and location. In accordance, another study that examined brain specimens from 47 patients with glioma indicated that PAX3 overexpression was positively associated with higher WHO grade, as evaluated by immunohistochemistry, irrespective to age, gender, tumor size, KPS score, as well as the presence of *IDH1* mutation or O-6-Methylguanine-DNA Methyltransferase *(MGMT)*

methylation. In addition, increased PAX3 levels were also correlated with poorer prognosis in this experiment [24]. Furthermore, it was demonstrated that the degree of PAX3 expression was stronger in 83 glioma tissues compared to 6 normal brain specimens, associated to tumor grade [41]. In agreement, the levels of miR-485-5p, which inhibits PAX3 expression, were found to be significantly decreased in high-grade glioma samples compared to low-grade [25].

Importantly, the prognostic value of PAX3 in gliomas was confirmed by another large recent study indicating that PAX3 was the most significant prognostic gene in glioblastoma patients, as assessed by whole genome mRNA expression array in 119 glioblastoma specimens in comparison to 5 normal brain tissues from the CGGA cohort [26]. More specifically, higher expression levels of PAX3 were associated with shorter survival time particularly of *IDH1* wild-type glioblastoma Chinese patients, independent of the various clinicopathological characteristics of the tumors. Interestingly, these results were not observed neither in the case of *IDH1* mutant carriers, nor in the Caucasian population from TCGA database, suggesting that race may substantially contribute to the molecular heterogeneity of gliomas [26]. These large CGGA and TCGA datasets allow the investigation of less-biased correlations between genetic factors and glioma prognosis, in comparison to the prognostic studies of specific sets of patients described above.

Collectively, these findings suggest that PAX3 could be of pivotal diagnostic and prognostic significance, representing a novel promising biomarker for gliomas that definitely needs to be further investigated.

PAX3 as a Potential Therapeutic Target in Gliomas
Chemotherapy is one of the most common treatment strategies against gliomas, whereas multidrug resistance represents a main reason for therapeutic failure. Therefore, elucidation of the molecular pathways involved in drug resistance is needed in order to overcome this important obstacle towards glioma treatment.

Interestingly, *PAX3* knockdown has been shown to significantly augment the cytotoxicity of etoposide, vincristine and cisplatin, three chemotherapeutic agents commonly used in neuroblastoma treatment [49]. Specifically in gliomas, *PAX3* knockdown *via* the use of siPAX3 was shown to significantly increase the cytotoxic effects of cisplatin, etoposide and vincristine in human U87 glioma cells in an additive manner, suggesting that simultaneous pharmaceutical inhibition of PAX3 expression could improve the efficacy of chemotherapy in gliomas [23]. Furthermore, hypoxia-induced undifferentiated state has been shown to contribute to glioblastoma chemoresistance *via* the upregulation of *Hypoxia-inducible factor 1-α (*HIF-1α) in BGSCs. Given the abovementioned role of PAX3 in BGSCs maintenance [50], we can speculate that this hypoxia-driven mechanism may play an important role in the observed increased chemoresistance by PAX3.

Apart from conventional chemotherapeutic agents, other novel treatment strategies are currently investigated for gliomas. In particular, combined inhibition of the *de novo* and salvage pathways of nucleoside synthesis has shown promising results in the case of acute lymphoblastic leukemia [51]. Interestingly, a recent study that used two compounds that selectively inhibit these pathways, named thymidine (dT) and DI-39 respectively, demonstrated that this combinatorial treatment induced differential responses in BGSCs across various subsets of human glioblastoma cell cultures [52]. Subsequent bioinformatics analysis indicated that resistance to therapy with dT and DI-39 was associated with increased PAX3

expression. Therefore, subclassification of glioblastoma cultures according to PAX3 expression would be of great importance in this treatment strategy, since it could distinguish sensitive from resistant ones. Furthermore, PAX3-co-targeting could probably significantly improve the efficacy of this novel therapeutic method.

Targeting BGSCs is one of the most promising therapeutic approaches against gliomas. Given the significant role of PAX3 in the maintenance of BGSCs stemness especially under hypoxic conditions, it has been proposed that oxygen tension should be taken significantly into account during BGSCs — and/or PAX3-targeted therapies [41].

Thus, apart from its pathogenic and prognostic significance, PAX3 may also represent an important therapeutic target against gliomas that deserves further study.

Concluding Remarks and Future Perspectives
In conclusion, although the subject is still in its infancy, there is a substantial growing body of evidence indicating the pivotal role of PAX3 in glioma development and progression, *via* its contribution to enhanced proliferation, increased invasiveness and resistance to apoptosis of glioma cells, as well as the maintenance of BGSCs stemness. Moreover, PAX3 could represent a novel diagnostic and prognostic biomarker for gliomas, facilitating future personalized treatment. Finally, PAX3 may contribute to the chemoresistance of glioma cells, and affect the effectiveness of other therapies that are currently under investigation.

It is important to mention that a considerable amount of evidence about the role of PAX3 in gliomas is derived from *in vitro* studies using cell lines involving U87, a human tumor-derived cell line. Of note, the authenticity and the extent to which this cell line has the identity of the actual tumor origin has been questioned, raising important issues regarding their clinical relevance to the field [53], since under serum culture conditions, genetic drift results in several U87 subclones that could affect experimental reproducibility [54]. On the other hand, NSCs can be stably maintained when cultured with growth factors without serum. In this regard, BGSCs, which may initiate gliomagenesis, promote tumor progression and induce resistance to chemotherapy and radiotherapy, have been proposed to represent a more appropriate experimental model for studying human GBM biology [54]. Nevertheless, results from preclinical studies using either U87 or BGSCs described above, confirm the significant role of PAX3 in glioma pathobiology.

Interestingly, given the well-established and important role of the *PAX3-forkhead box O1 (FOXO1)* fusion gene in rhabdomyosarcoma [55], its ability to excessively trigger *PAX3* gene expression and the fact that *PAX3-FOXO1* targeting approaches are currently under investigation [56], a probable future field of research might be the potentially pathogenic role of this fusion gene in gliomas. Nevertheless, the specific underlying mechanisms involved in the oncogenic properties of PAX3 need to be more deeply elucidated, in order to develop more effective treatment approaches.

Conflict of Interest
All the authors declare no potential conflict of interest.

Authors' Contribution

EA carried out the literature review, conceptualized, and drafted the manuscript. YNP revised and edited the manuscript. CP provided critical inputs, revised and edited the final version of the manuscript. All authors read and approved the final version of the manuscript.

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