Transforming growth factor β and bone morphogenetic protein actions in brain tumors

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1. TGF-β and BMP actions in cancer

The largest family of developmental polypeptide growth factors is the transforming growth factor β (TGF-β) family. TGF-β family members score prominently in other human diseases, beyond cancer, because these factors are resident in a dormant state in the extracellular environment of adult tissues and their functions become activated every time tissues get wounded or inflamed. In addition, the pathogenic activation of the TGF-β family leads to an imminent cascade of synthesis, secretion and activation of the same and many of its sister growth factors in the family, leading to positive auto-regulatory loops that sustain growth factor activity over time and contribute to disease progression. Local tissue residence of TGF-β family members aims at the maintenance of organ homeostasis; when tumor development proceeds, cancer cells succeed in inactivating the function of the TGF-β pathways by genetically altering some key molecules. Induction of cytostasis and apoptosis by TGF-β family members contributes to the homeostatic control, whereas the sustained auto-inductive cycles of these cytokines contributes to their abundant presence in all cancers and their subsequent contribution to cancer cell differentiation, neo-angiogenic stimulation and suppression of immune surveillance by resident cells in the developing cancer microenvironment.

The signaling pathways that mediate physiological and pathological effects of the TGF-β family are highly conserved in all metazoan organisms and involve the type II and type I receptors that form heterotetrameric complexes on the cell surface and bind the dimeric ligands, initiating signaling by the protein kinases of the two receptor types. Using a sequential mechanism, ligand-bound type II receptor recruits and phosphorylates the type I receptor, which then recruits and phosphorylates Smad family proteins, the Smad1, Smad5 and Smad8 in the BMP sub-family and the Smad2 and Smad3 in the TGF-β sub-family, all five of which can pair with Smad4, a family member that is...
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not phosphorylated by the type I receptor. The receptor complex also recruits adaptor proteins and via post-translational modifications, activates several signaling proteins that transmit biological information in parallel to the receptor-phosphorylated Smads. These signaling proteins are ubiquitin ligases, protein and lipid kinases, and small GTPases [1,3]. The integrated signaling input of all these proteins orchestrates a genomic response that is tissue-specific and developmental stage- or pathogenic state-specific and explains the impact TGF-β members have on various biological processes (Fig. 1) [1,3]. In the context of cancer, physiological signaling mechanisms maintain their components but seem to operate in a perturbed manner, sometimes due to the absence or excessive abundance and overactivity of regulatory proteins, and sometimes due to complete lack of specific key mediators of the pathways, such as receptors, co-receptors or Smad proteins. An interesting fact is the genetic inactivation of TGF-β family signaling proteins that occurs in a tissue- or organ-specific manner. In other words, whereas colorectal cancer preferentially mutates the TGF-β type II receptor or Smad4 genes, and pancreatic cancer almost universally mutates the Smad4 gene, breast cancer rarely if ever, mutates genes in these pathways [4,5]. Brain malignancies develop in a manner that strongly depends on the action of TGF-βs and BMPs in the tumor microenvironment [6,7]. However, brain tumor cells mutate genes of these pathways only with relatively low frequency [8].

In this article, we discuss brain malignancies from the perspective of the TGF-β family, and due to space limitation we focus more on the actions of the prototype members, TGF-βs and some of the BMPs. We aim at comparing signaling mechanisms and coordination of cellular activities that contribute to the progression of the disease. We highlight knowledge on cancer stem cells (CSCs) and also discuss the problem of invasiveness in brain cancer. We finally touch upon the prospect of therapy that is based on the basic understanding of the function of TGF-β pathways.

2. Brain malignancy and the TGF-β family

There are several types of central nervous system (CNS) malignancies which are scaled between I and IV based on histologic features of the tumor, according to the World Health Organization (WHO). As a general rule, patients with grade II tumors survive more than 5 years, patients with grade III tumors survive between 2 and 3 years; patients with grade IV tumors have the worst prognosis, which also depends on the type of malignancy they are diagnosed with. For example, the grade IV brain tumor with the least survival time after its diagnosis is glioblastoma multiforme (GBM) with a median survival of 1 year, whereas cerebellar medulloblastoma, another grade IV brain tumor, if treated, can show a 5-year survival rate of 60–80% [9]. Gliomas are the most common brain tumors, accounting for 80% of all CNS tumors; the most aggressive form of gliomas is GBM. Here we will focus on those brain tumors in which it has been reported that the TGF-β family affects their initiation, growth and therapy response.

A hallmark feature of the association of cytokines like TGF-β with the development of cancer is their detection and abundance in the bloodstream of patients. For example, a correlation between high TGF-β2 levels in the plasma of patients and their advanced high-grade glioma and a poor prognosis has been reported [10]. Interestingly, the plasma levels of TGF-β2 were predicted to change in patients receiving tamoxifen in their therapeutic cocktail, a hypothesis proposed based on breast cancer studies [11]. However, in vivo studies in GBM patients could not reveal a good correlation between TGF-β2 plasma levels and the patient response to therapy, whereas in vitro, GBM cell lines cultured in the presence of tamoxifen did in fact present enhanced secretion of TGF-β2. Similar to TGF-β2, studies on the prognostic value of plasma levels of the mature and latent forms of TGF-β1 also failed to reveal a clear correlation with prognostic significance in GBM patients treated with radiation [12]. Despite the results of these earlier studies, more recently, expression of both TGF-β1 and TGF-β2 was found to be higher in GBM compared to healthy brain tissue, and the higher the expression of these two ligands, the worse the prognosis for the patient [13]. Genome-wide expression analyses in blood vessels after microdissection of the vascular cells from GBM patient biopsies, identified TGF-β2 as a key mediator of the dramatic neo-angiogenesis observed in the tumors of these patients, and an overall enhanced Smad activity signature was one of the key findings of this study [14]. It is worth noting that detection of TGF-βs in biological fluids has always been difficult and the technology and sensitivity of detection has clearly been improved in the recent years. A member of the TGF-β family with clear association with the progression of brain malignancy, and undisputable prognostic value, is GDF15 [15]. Measurement of GDF15 in the cerebrospinal fluid provided more reliable results when compared to the levels of GDF15 in the plasma, and clearly patients with GBM and meningioma that scored high in the GDF15 scale exhibited a shorter survival [15]. On the other hand, BMP4 seems to be a predictor of good prognosis in gliomas [16]. The same has been established for BMP2 in GBMs and lower grade gliomas [17]. These data already underscore that TGF-β and BMP tend to have opposite roles in brain tumor development and prognosis.
Studies of TGF-β family members in patient plasma or cerebrospinal fluid are usually accompanied by in vitro studies of human brain tumor cell lines and mouse models of brain malignancy [6,7]. Interestingly, TGF-β2, although originally identified in bovine bone, was also molecularly cloned as a secreted factor by GBMs [18], which spurred strong interest in the roles of the TGF-β family in brain malignancy from the very early days of the TGF-β field.

In vitro analyses of the proliferative responses of normal astrocytes compared to GBM cells, explained that GBM cells retain many responses to TGF-β, such as extracellular matrix synthesis and pro-angiogenic factor secretion, whereas GBMs lack the anti-proliferative response to TGF-β, partly due to the genetic loss of the cell cycle inhibitor gene p15 Ink4b [19]. The cell cycle inhibitors p15 Ink4b and p21 Cip1 are major downstream effectors of TGF-β-mediated cytostasis, and GBMs selectively express transcription factors like FoxG1, that bypass the normal control of these genes by the TGF-β receptor-activated Smad signaling [20]. Another mechanism that helps GBM cells bypass normal astrocyte cell cycle control by TGF-β involves the phosphatase and tensin homologue deleted on chromosome 19 (PTEN), which is frequently inactivated in GBMs [21]. PTEN associates with Smad3 and down-regulates its transcriptional activity, whereas loss of PTEN in GBMs provides enhanced Smad3-dependent activity that renders TGF-β pro-invasive and pro-tumorigenic. After this brief and selective presentation of processes that link TGF-β function to malignant phenotypes in the brain, we will proceed with a more systematic analysis of the role of this family of cytokines in various key anatomical and functional compartments of brain tumors.

3. Brain tumor stem cells

Glioblastoma is the most aggressive type of CNS tumors; despite several treatment strategies such as surgery, radiotherapy and chemotherapy, the median survival is 1 year after diagnosis, and this is thought to be due to the resistance and power of CSCs. More than a decade ago the existence of CSCs in GBMs and medulloblastoma was proven; such CSCs were identified as tumor cells that express the CD133 protein on their surface, and transplantation of as few as 100 CD133+ cells were sufficient in recapitulating the heterogeneity of the original tumor [22]. GBM CSCs (G-CSCs) are multipotent, have the property of self-renewal and are thought to be responsible for tumor maintenance, recurrence and therapy resistance [22–25]. Several mouse models have provided evidence that normal neural stem and progenitor cells give rise to malignant astrocytomas [26–28]. Similar to neural stem cells (NSC), G-CSCs also give rise to more differentiated cells, the bulk of the tumor, which is highly heterogenic and less tumorigenic (Fig. 2). Interestingly, both NSCs and G-CSCs are controlled by similar signaling pathways involved in neurogenesis and brain formation, such as Notch, Sonic hedgehog (Shh), Wnt and the TGF-β family [29].

3.1. TGF-β actions in brain CSCs

TGF-β is known to have cytostatic effects inducing the expression of p21Cip1 in epithelial cells as mentioned above; however, in both neuroepithelial and glioblastoma cells, the levels of the transcription factor FoxG1 are higher, causing this protein to
directly interact with FoxO3, and thus inhibiting its capacity to promote p21^{Cip1} expression in response to TGF-β [20]. This mechanism explains how GBMs can resist to cytostatic TGF-β, and switch their response to this cytokine, thus promoting cell proliferation. A clear correlation between poor survival and high levels of phospho-Smad2 has been demonstrated; high levels of proliferating cells correlated with high levels of phospho-Smad2 in GBM patients, indicating a positive role of TGF-β in GBM cell proliferation [30]. According to this work, TGF-β promotes proliferation of glioma cells by inducing the expression of platelet-derived growth factor (PDGF)-BB in a Smad2/3-dependent manner. Interestingly, another report proposed that transcription factor Olig1 is also involved in PDGF-BB induction in response to TGF-β, whereas the transcriptional regulator human homologue of Maid (HHM) could counteract the positive effect of Olig1 on the transcriptional regulation of PDGF-BB by Smad2/3 [31]. In addition, TGF-β can promote the growth of glioma cells and inhibit apoptosis by inducing the expression of another TGF-β family member, Nodal; interestingly, Nodal expression in grade IV glioma correlates to the invasive potential of tumor cells in an in vivo model as well as in a patient cohort [32,33]. TGF-β1 can also induce the expression of TGF-β2 in GBM cell lines in a CREB1- and Smad2/3-dependent manner [13,34], and knockdown of CREB1 results in fewer tumors and longer survival. Another pathway through which TGF-β promotes the aggressiveness of glioma is by sustained activation of nuclear factor of κ light polypeptide gene enhancer in B-cells (NF-κB), via upregulation of the microRNA miR-182, which suppresses the cylindromatosis protein Cyld, an established negative regulator of NF-κB signaling [35].

Autocrine production of TGF-β is necessary for G-CSCs to maintain their stemness, which acts via different downstream pathways (Fig. 2). TGF-β up-regulates the expression of the stem cell transcription factor Sox2 through its sibling, Sox4, in a Smad2/3-dependent manner; in agreement, inhibition of TGF-β receptors results in down-regulation of the expression of Sox4 and Sox2, and decreased self-renewal capacity as well as down-regulation of stem cell markers, such as CD133, nestin and Musashi [36]. TGF-β, in a Smad2/3-dependent manner, also induces the expression of the cytokine leukemia inhibitory factor (LIF) in order to enhance G-CSC stemness, and treatment of G-CSCs with TGF-β or LIF in vitro, increases their self-renewal capacity and their tumor-initiating potential in vivo [37]. TGF-β increases the numbers of G-CSCs that are positive for the surface protein CD44 and also induces the expression of the transcriptional regulators Id1 and Id3; in contrast, inhibition of TGF-β receptors in G-CSCs reduces their self-renewal capacity by diminishing the G-CSC subpopulation of CD44_{high}/Id1_{high} cells, which results in a decrease of their tumor-initiating capacity and oncogenic potential [38]. Interestingly, Id1, Id3, LIF, Sox2 and Sox4 are all necessary to maintain the CD44_{high} population, indicating that all these molecular pathways are interconnected. However, not all G-CSCs respond potently to TGF-β and this can be explained by the genetic background of GBMs in different patients. For example, a small percentage of GBM patients show amplification of the USP15 (ubiquitin specific peptidase 15) gene, coding for a deubiquitinating enzyme, which deubiquititates and stabilizes the TGF-β type I receptor, leading to enhanced TGF-β activity in those tumors and promoting their tumor-initiating capacities [39]. Obviously, GBM patients with low or no activity of USP15 would suffer from relative resistance to TGF-β.

3.2. BMP actions in brain CSCs

In contrast to the positive effects that TGF-β exerts on G-CSC stemness, BMPs have been proven to have opposite functions (Fig. 2). First, it was published that treatment with BMP4 decreases the percentage of CD133^{+} cells in G-CSCs, and promotes their differentiation mainly towards astrocytes, and to a lesser extent

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**Fig. 2. Opposing actions of TGF-β and BMP signaling in glioblastoma multiforme cancer stem cells.** A brain tumor made of its heterogeneous differentiated cells (shown in pink and green) and their associated tumor microenvironment is schematically drawn. CSCs (yellow) produce TGF-β and BMPs to promote their differentiation towards several lineages depending on the brain tumor type. Finally, tumor cell migration can be promoted by TGF-β, BMPs and several EMT-TFs.
towards the neuronal and oligodendroglial lineages [40]. BMP4 could also reduce GBM cell proliferation. In agreement with these results, a BMP7 variant could also reduce G-CSC proliferation and induce their differentiation towards astrocytes and neurons both in vitro and in vivo [41]. We demonstrated that in order for BMP7 to promote GBM differentiation, up-regulation of the transcription factor Snail is required, and the sole overexpression of Snail could mimic many of the effects of BMP7 treatment both in vitro and in vivo [42]. All these data indicate that BMPs act as tumor suppressors in GBM. Moreover, in a subset of GBM patients, the type I receptor gene BMPRIB is hypermethylated by the Polycomb group histone methyltransferase EZH2, rendering these tumors resistant to BMP-induced differentiation [43]. In agreement with this mechanism, another subunit of the Polycomb group complex, the stem cell factor BM1 has been shown to suppress genes like the transcription factor ATF3 (activating transcription factor 3), which limits GBM cell responsiveness to BMP signaling and enhances GBM responsiveness to TGF-β signaling [44]. GBMs that inactivate the function of BM1 then switch their responsiveness and become hypersensitive to the differentiating potential of BMP, while losing their sensitivity to TGF-β. The specific mechanisms by which ATF3 would protect GBMs from acquiring stem-like and self-renewing features may involve the transcriptional activity of ATF3 complexes with other components of the activating protein 1 (AP-1) family of transcription factors, and requires further deeper understanding [44]. Another mechanism by which G-CSCs bypass BMP-induced differentiation is the high expression levels of the extracellular BMP antagonist Gremlin [45]. A G-CSC sub-population expresses higher levels of Gremlin compared to non-G-CSC tumor cells, and overexpression of Gremlin in the non-G-CSC cells was sufficient to shift them towards a G-CSC phenotype resulting in enhancement of their tumor-initiating properties (Fig. 2). In contrast to the previous results, it has also been described that BMP2 can promote GBM proliferation, migration, enhanced self-renewal of G-CSCs in vitro and enhanced tumor formation in vivo [46]. Sensitivity of the GBMs to BMP2 is further secured due to downregulation of miR-656, which targets the type I receptor BMPR1A and when highly expressed, miR-656 can impair BMP2 effects [46].

4. TGF-β and BMP actions in other brain tumors

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, and according to histopathological characteristics, five subgroups of MB are currently classified by WHO; however, according to classification based on MB gene expression profiles, only four subgroups can be recognized, including genes that exhibit alterations in the (1) Wnt pathway, (2) Shh pathway, (3) Myc signaling and (4) undefined genetic processes [47]. Activation of Shh promotes proliferation in MBs and BMP2 can block Shh-induced proliferation by upregulating the expression of the zinc finger transcription factor TIEG1 (TGF-β immediate-early gene 1), which can repress N-Myc transcription [48]. BMP2 can also promote differentiation of cerebellar granular neuronal precursors (CGNPs) towards neurons [48]. Another mechanism through which BMP2 and BMP4 inhibit CGNP proliferation and induce their differentiation is by rapid proteasome-mediated degradation of Atoto1/Math1, a BHLH transcription factor required for cerebellar development [49]. BMP2 has also been described to induce apoptosis in MBs [50]. In contrast to the Shh subgroup of MB, in the Myc subgroup, it has been described that Myc promotes BMP7 expression which is required for MB cell proliferation and survival [51]. A large screen of MB patients belonging to the Myc group showed that 20% of the cases were enriched in expression of TGF-β pathway-related genes; in addition, the type II activin receptors ACVR2A and ACVR2B and the TGF-β type I receptor TGBR1 were highly amplified, which suggested possible new therapeutic targets in this subgroup of MB, characterized by its very poor prognosis [52]. In another report, opposing results were described, showing that patient samples with positive nuclear staining of Smad3 correlated with a survival advantage in these patients [53]; however, in this study, the patient subgroup 3 (Myc) was under-represented, which might explain the discrepancy between the last two studies.

Diffusive high-grade gliomas (HGGs) that develop during childhood have a very poor survival: when the tumors arise in the cerebellar cortex, the 2-year survival chance of HGG patients is 30%; when the tumors arise in the brain-stem they are called diffuse intrinsic pontine gliomas (DIPGs) and their 2-year survival chance is less than 10% [54]. High grade tumors share similar histopathology and gene expression subgroups between adult glioblastoma and childhood HGGs are also similar. However, recent publications have shed light to the more rare tumors of children as being unique in terms of the molecular process that drives their evolution, depending on the genetic mutations acquired, the anatomical regions were tumors form, and the age group. Thus, recent evidence showed that the BMP type I receptor known as activin receptor type I (ACVR1 or activin-receptor-like kinase 2 (ALK2)) is mutated in 24% of DIPG patients but not in paediatric NBS-HGGs. ACVR1-mutant DIPGs occur earlier in age, have a longer survival and are more frequent in females. The mutations identified are p.Arg206His, p.Gly328Glu and p.Gly356Asp, located within the serine/threonine kinase domain or the glycine-serine (GS) rich-domain of this type 1 receptor kinase, which is expected to shift the kinase to an active conformation, and results in an increased phosphorylation of Smad1/5/8 in the studied tumors. The same ACVR1/ALK2 mutations found in DIPG are also found in patients with the syndrome fibrodysplasia ossificans progressiva (FOP); however, the FOP patients are not predisposed to cancer, which may reflect that ACVR1 mutations are not related to tumor-initiating capacity in DIPG, but rather give an advantage to tumor cells when mutations in different pathways coexist with the ACVR1 mutations [55-58]. Patients with mutation in the histone H3 gene (H3F3A) exhibited phosphorylated levels of Smad1/5/8 even though they had wild-type ACVR1, suggesting additional ways of BMP activation in DIPG [57]. However, further studies are needed to validate whether ACVR1 can be a useful therapeutic target in DIPG, and to understand its role in tumorigenesis.

Furthermore, in pediatric HGG precursors, it has been described that oxygen levels modulate the BMP2 response: BMP2 induces astrogial differentiation, but hypoxia can block this effect; hypoxia can also attenuate the anti-proliferative effects of BMP2. Interestingly, BMP2 can downregulate hypoxia-inducible factor α (HIFα) under hypoxic conditions in paediatric HGG but not in normal subventricular cells [59]. Finally, the effects of BMPs in oligodendroglioma propagating cells were recently characterized, whereby BMPs decreased cell proliferation, depleted the CD133+ population, and promoted astrocytic differentiation, probably by cytoplasmic sequestration of Olig1 and Olig2 by their inhibitory partners, Id2 and Id4 [60].

Overall, the majority of published work indicates that BMP pathways play pro-differentiation and anti-proliferative roles in most brain tumors, except in DIPGs; in contrast, TGF-β mostly plays pro-tumorigenic roles and promotes stemness and self-renewal (Fig. 2).

5. Brain tumor invasiveness

The invasive nature of brain tumors plays an important role in the ineffectiveness of surgery and is one of the causes behind their...
poor prognosis and relapse. Moreover, some anti-angiogenic treatments can unfortunately enhance glioma invasiveness [61]. The pattern of glioma cell migration follows the pattern of glial progenitor cells during normal brain development, and in both cases it is controlled by the local microenvironment. Microglia has been proven to produce TGF-β that in turn promotes cell proliferation and migration in GBM cells [62]. The integrin-β3 is known to activate the latent form of TGF-β1 in neural stem cells and it is also important to regulate the invasiveness of GBM-perivascular tumor cells [63,64]. TGF-β2 promotes glioma cell migration by enhancing matrix metalloprotease 2 (MMP2) expression and its activity, as well as by inducing the expression of integrin-αv,β3 [65,66].

Glioblastomas have been divided into three main subgroups according to their molecular profile, the proneural (tumors express genes typical of neural progenitor cells), classical (they express genes related to proliferation and receptor tyrosine kinase activation), or mesenchymal (they express genes related to mesenchymal tissues) [67]. GBMs of the mesenchymal subgroup have worse prognosis in comparison with proneural tumors [68]. TGF-β is known to induce an epithelial to mesenchymal transition (EMT) in epithelial cancers giving rise to more motile and invasive cells, which have acquired mesenchymal characteristics both at the molecular and morphological level [69]. TGF-β regulates a network of embryonic transcription factors in order to induce EMT: Snail, Slug, Twist, ZEB1, and ZEB2, known as EMT-Transcription Factors (EMT-TFs). The chromatin factor high mobility group A2 (HMGA2) is an immediate-early gene of TGF-β (EMT-TFs). The chromatin factor high mobility group A2 (HMGA2) is an immediate-early gene of TGF-β (EMT-TFs). The chromatin factor high mobility group A2 (HMGA2) is an immediate-early gene of TGF-β (EMT-TFs). The chromatin factor high mobility group A2 (HMGA2) is an immediate-early gene of TGF-β (EMT-TFs).

6. Roles of TGF-β and BMP in the tumor microenvironment

In the last decade, the importance of the tumor microenvironment in tumor progression and its role as the niche for CSCs has surfaced. G-CSCs have been described to reside near vascularized areas within the tumor [83]. Not only do G-CSCs take advantage of the existing vasculature, but they also induce angiogenesis [84], plus they can generate vascular pericytes in order to support the structure and function of the vasculature; most of GBM pericytes are derived from neoplastic cells (Fig. 2). In part this trans-differentiation is driven by TGF-β produced by endothelial cells (ECs) acting on the G-CSCs [85]. At the same time, TGF-β secreted from GBM cells can affect ECs and promote angiogenesis by increasing the expression of the insulin-like growth factor-binding protein 7 (IGFBP7) [86]. Similarly, EC-secreted TGF-β induces the expression of the mesenchymal cadherin-11 in nearby GBM cells, enhancing their invasive potential, a process that mimics embryonic exit and migration of neuroepithelial cells from the ventricular zone [87].

Glioma-associated microglia and macrophages (GAMs) can contribute up to 30% of the mass of a brain tumor. G-CSCs recruit GAMs into the tumor and promote their shift towards M2 macrophages (Fig. 2). TGF-β is known to be able to promote the M2 shift in other tumors, but in glioma it has not been properly proven. The tumor-promoting M2 macrophages are characterized by having diminished capacity to induce an anti-tumor T cell response, are able to mediate immune suppression and produce several factors that stimulate glioma growth, neovascularization and invasiveness [88]. On one hand, tumor-derived TGF-β can suppress the activation and proliferation of microglia [89]. On the other hand, microglia ablation results in reduction of glioma size and improved survival [90] as well as reduced migration [91], indicating the importance of microglial cells in glioma progression. One of the mechanisms through which microglia exerts its pro-invasive functions is by secreting TGF-β [62]. Moreover, tumor-associated microglia/macrophages enhance G-CSC invasion through their production of TGF-β1 that enhances MMP9 expression in CD133+ GBM cells [92]. Another immune cell type present in the microenvironment of gliomas is myeloid-derived suppressor cells (MDSCs), which is a heterogeneous population of activated immature cells
myeloid precursors consisting of dendritic cells, macrophages and granulocytes, which can cause immune suppression (Fig. 2). MDSCs among other cytokines secrete TGF-β1 which might contribute to their immunosuppressive effects [93].

TGF-β has been described to promote recruitment and/or expansion of regulatory T cells (Treg) in gliomas, as systemic administration of the anti-TGF-β1 mAb, 1D11, results in a decreased infiltration of Treg in these tumors [94]. In high-grade glioma, an abundant population of IL-17+ Tregs has been described and found responsible for suppressing CD8+ T cell proliferation in a TGF-β1-dependent manner [95] (Fig. 2). Moreover, another mechanism through which TGF-β exerts its immunosuppressive role is by downregulating the expression of the activating receptor Th1/2 in glioma cells promotes their recognition by CD8+ T and NK cells [96,97]. Finally, TGF-β silencing, as mentioned in the previous section, resulted in loss of migratory and invasive capacities by glioma cells [97]. TGF-β1 and TGF-β2, by reducing the expression of the adhesion proteins ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1) in GBM-associated ECs, can reduce T cell transmigration, suggesting that TGF-β blockade would result in improved intratumoral T cell infiltration and better response to immunotherapies [98]. In a medulloblastoma mouse model, it has been observed that blocking the TGF-β pathway in T cells (both CD4+ and CD8+ populations) results in slower progression of the disease, reduced Treg infiltration and promoted CD8+ T cell differentiation into CD8+ cytotoxic T lymphocytes conferring antitumor immunity [99]. All together the evidence indicates that TGF-β1 in glioma and other brain tumors has a potent immunosuppressive role and blockade of the TGF-β pathway will not only affect the G-CSC population and tumor cell invasive capacity, but will also improve the capacity of the immune system to play its anti-tumor activities.

Finally, glioblastoma cells can also attract and interact with neural precursor cells (NPCs) [100]. NPCs can secrete several molecules, among them BMP7 which can promote G-CSC differentiation and play a tumor suppressor role [101] (Fig. 2); this anti-tumor response is much more potent in young compared to old mice.

7. Therapeutic perspectives

7.1. BMP-based therapy

As introduced earlier, the fact that GBM accounts for 52% of primary brain malignancies [102], and its characteristic poor survival (12–15 months with multimodal therapy), poor treatability, frequent relapse and acquired chemoresistance to temozolomide [103], make the prospect of new therapies against GBM a task of imminent importance. Moreover, GBM has a poor metastatic outspread but significant infiltration into the brain and spinal chord, factors that hinder its surgical resection. All these features contribute to define GBM as a hard to treat and difficult to cure malignancy, based on the current chemotherapeutic, radiotherapeutic and surgical resection approaches. It is therefore considered important to elucidate new effective strategies for GBM treatment, which can lead to its complete regression. It has been addressed how future strategies might encompass different routes: targeting the vascular niche or the therapeutic resistance of G-CSCs, blocking G-CSCs via their specific markers (CD133 or LiCAM (Li cell adhesion molecule)), modulating signaling pathways that are abnormally regulated in the G-CSC population -such as Notch, BMP, TGF-β, STAT3 or Wnt- targeting transcription factors or miRNAs that are differentially expressed in or affect the G-CSC population by forcing cell differentiation [103].

In this article, we have explained how the misregulation of TGF-β and BMP pathways can contribute to GBM progression, either by influencing invasiveness and the angiogenic process, or by acting on the stem cell niche and the stromal cell pool. Thus, recent studies have focused on understanding how these pathways can be modulated in order to increase the effectiveness of GBM treatment. For example, BMP2 could sensitize G-CSCs to temozolomide by blocking the HIF1α/MGMT pathway, involved in chemoresistance acquisition [104]. In particular, once highly tumorigenic and core tumor layer-derived G-CSCs are pretreated with BMP2, they become more sensitive to the cytotoxic effect of TMZ, with consequent depletion in the stem niche and induction of differentiation of live remaining G-CSCs. This study also elucidated how BMP2/TMZ can downregulate HIF1α and the HIF1α-dependent MGMT expression, thus reducing GBM chemoresistance [104]. Furthermore, it has recently been reported how BMP4-engineered vaccinia viral particles induce G-CSC growth inhibition, loss of stemness and acquisition of differentiation features in vitro. BMP4-producing vaccinia viruses were then delivered intracranially in an orthotopic mouse model of GBM, thus leading to an improved tumor regression, lower tumor burden and better survival in vivo [105]. In addition, the importance of blocking the BMP9 pathway during angiogenesis via the use of the small molecule kinase inhibitor K02288 binding to the endothelial type I receptor ALK1 has been defined [106]. This inhibitor decreased both Smad- and Notch-dependent responses, causing hypersprouting of the vessels and dysfunctional angiogenesis in vitro, which might be of interest considering the florid neoangiogenesis in GBM [106]. Intriguingly, a biodegradable device that provides controlled release of bioactive BMP7 in vitro has been described, where BMP7 was encapsulated in a heparin core and surrounded by a shield of biodegradable polyester matrix [107]. This nano-system was able to release bioactive BMP7 in a controlled fashion for 2 months inhibiting neurosphere formation and cell proliferation in vitro [107].

7.2. TGF-β-based therapy

Targeting also the TGF-β pathway in GBM might be a promising strategy of treatment. As an example, the inhibition of TGF-β2 -the most abundant TGF-β isoform in GBM- with the antisense oligonucleotide AP12009 was associated with prolonged survival in three phase I/II studies on patients with recurrent or refractory high-grade gliomas and complete tumor remission in two patients [108]. Additionally, the TGF-βRI kinase inhibitor LY2109761 was found to decrease clonogenicity, enhance apoptosis and promote radiosensitiveness in GBM cell lines, while after intracranial administration it was associated with reduced tumor growth, invasion and neoangiogenesis and prolonged survival in a GMB xenograft model [109]. The positive correlation between an active TGF-β signaling and GBM radiosensitivity was pointed out also in another study, which clarifies how inhibiting the TGF-β pathway with the TGF-βRII kinase inhibitor LY364947 or a pan-TGF-β neutralizing antibody in vitro, can sensitize GBM cells to ionizing radiation due to reduction of the DNA damage response [110]. This study also demonstrated how TGF-β inhibition in conjunction with radiation was able to decrease neurosphere forming ability in vitro [110]. A study of dose escalation of the TGF-βRII kinase inhibitor LY2157299 monohydrate in patients has lately been reported, which was assessed to be safe – without any signs of cardiac toxicity – and effective for patients, with a partial or complete response in 21% of the cohort members [111]. Interestingly, TGF-β can also affect other signaling pathways, such as vascular endothelial growth factor (VEGF), in order to promote acquired chemoresistance. For example, the use of VEGF inhibitors can paradoxically induce TGF-β signaling, which leads to
expression of the chemokine receptor CXCR4 and its ligand CXCL12. This promotes GBM invasiveness and suggests that blocking the TGF-β pathway, might be a promising adjuvant solution to current radiotherapy regiments [112]. All examples of therapy are derived from studies in GBM, as therapeutic approaches of other types of brain malignancy have not yet been based on the manipulation of the TGF-β and BMP pathways. The new examples of DIPG suggest that focus should be addressed to the inhibition of the ACVR1/ALK2 receptor, an area that will most certainly thrive in the near future. Taken together, TGF-β and BMPs can be considered potentially interesting pathways to modulate in order to enhance the efficiency of current chemotherapeutic and radiotherapeutic approaches in malignancies of the brain.

8. Conclusions and perspectives

In conclusion, TGF-β family members are important players in brain tumor progression. TGF-β1 and TGF-β2 are key factors that promote CSC self-renewal, tumor cell migration and that repress the immune system. In contrast, BMPs in most brain tumors promote CSC differentiation and block proliferation. However, BMPs can also enhance tumor cell migration, and their role in the brain tumor immune response is not well studied. TGF-β and BMP pathways are well studied, however, their use as either therapeutic targets or agents respectively have still not proven as effective as expected. This is probably due to the signaling complexity of the TGF-β family. The urgent need for combinatorial therapies to improve patient survival and block tumor recurrence suggests that anti-TGF-β and pro-BMP agents can be used in combination with other drugs to enhance the efficacy of therapy in malignancies of the brain.

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