FEBS Letters 589 (2015) 1588–1597

journal homepage: www.FEBSLetters.org

Review

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

Laia Caja, Claudia Bellomo, Aristidis Moustakas *

Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Box 582, SE-751 23 Uppsala, Sweden
Ludwig Cancer Research, Science for Life Laboratory, Uppsala University, Box 595, SE-751 24 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 29 March 2015
Revised 28 April 2015
Accepted 29 April 2015
Available online 6 May 2015

Edited by Wilhelm Just

Keywords:
Bone morphogenetic protein
Brain
Cancer
Differentiation
Glioma
Transforming growth factor β

ABSTRACT

Members of the transforming growth factor β (TGF-β) family are implicated in the biology of several cancers. Here we focus on malignancies of the brain and examine the TGFβ and the bone morphogenetic protein (BMP) signaling branches of the family. These pathways exhibit context-dependent actions during tumorigenesis, acting either as tumor suppressors or as pro-tumorigenic agents. In the brain, the TGF-βs associate with oncogenic development and progression to the more malignant state. Inversely, the BMPs suppress tumorigenic potential by acting as agents that induce tumor cell differentiation. The latter has been best demonstrated in grade IV astrocytomas, otherwise known as glioblastoma multiforme. We discuss how the actions of TGF-βs and BMPs on cancer stem cells may explain their effects on tumor progression, and try to highlight intricate mechanisms that may link tumor cell differentiation to invasion. The focus on TGF-β and BMP and their actions in brain malignancies provides a rich territory for mechanistic understanding of tumor heterogeneity and suggests ways for improved therapeutic intervention, currently being addressed by clinical trials.

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 that includes thirty-three human genes encoding for biologically important proteins such as activins, bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs), which control embryonic development, organogenesis and adult organ homeostasis [1,2]. TGF-β was originally discovered as an inducer of oncogenic transformation and today we appreciate its complex role in cancer progression, which is characterized by the parallel or sequential involvement of many of its family members in the evolution of a given cancer [3]. TGF-β 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 [4]. In addition, the pathogenic activation of the TGF-βs 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 [4]. 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 [5]. 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 dedifferentiation, neo-angiogenic stimulation and suppression of immune surveillance by resident cells in the developing cancer microenvironment [4,5].

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 (Table 1) [1,3]. 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

* Corresponding author at: Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Box 582, SE-751 23 Uppsala, Sweden. Fax: +46 18 160420.
E-mail address: aris.moustakas@imbim.uu.se (A. Moustakas).

http://dx.doi.org/10.1016/j.febslet.2015.04.058
0014-5793/

Table 1
TGF-β family ligands and receptors.

Ligand	Type I receptor	Type II receptor
TGF-β1	TGFβRI/ALK5, ActRIA/ALK1	TGFβRII
TGF-β2	TGFβRI/ALK5, ActRIA/ALK1	TGFβRII
TGF-β3	TGFβRI/ALK5, ActRIA/ALK1	TGFβRII
ActivinβA	ActRIB/ALK4	ActRIIA, ActRIIB
ActivinβB	ActRIB/ALK4, ActRIC/ALK7	ActRIIA, ActRIIB
GDF1	ActRIB/ALK4, ActRIC/ALK7	ActRIIA, ActRIIB
GDF3	ActRIB/ALK4, ActRIC/ALK7	ActRIIA, ActRIIB
Nodal	ActRIB/ALK4, ActRIC/ALK7	ActRIIA, ActRIIB
BMP3B/GDF10	ActRIB/ALK4	ActRIIA,
GDF11	TGFβRI/ALK5, ActRIB/ALK4	ActRIIA, ActRIIB
GDF8/myostatin	TGFβRI/ALK5, ActRIB/ALK4	ActRIIB
GDF9a	TGFβRI/ALK5, ActRIB/ALK4, ActRIC/ALK7	ActRIIB
GDF9b		BMPRII
BMP9	ActRIA/ALK1	ActRIIA, ActRIIB, BMPRII
BMP10	ActRIA/ALK1	ActRIIA, ActRIIB, BMPRII
BMP2	BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
BMP4	BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
GDF5	BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
GDF6	BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
GDF7	BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
BMP5	ActRIA/ALK2, BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
BMP6	ActRIA/ALK2, BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
BMP7	ActRIA/ALK2, BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
BMP8A	ActRIA/ALK2, BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
BMP8B	ActRIA/ALK2, BMPRIA/ALK3, BMPRIB/ALK6	ActRIIA, ActRIIB, BMPRII
GDF15	BMPRIA/ALK3 (?)	ActRIIB (?)
BMP15	BMPRIB/ALK6	BMPRII
AMH	ActRIA/ALK2, BMPRIA/ALK3	AMHRII

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.

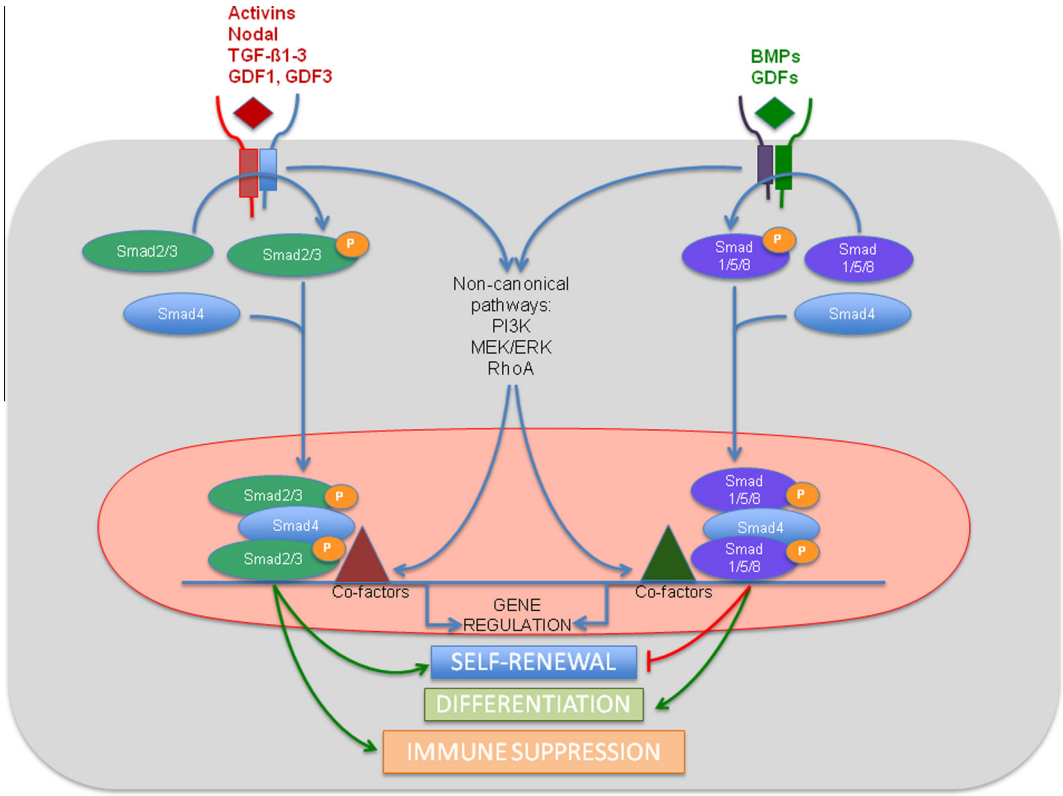

Fig. 1. Schematic representation of TGF-β and BMP signaling. Upon ligand binding to type II receptors, they form a heterotetrameric complex with the type I receptors, which then phosphorylate the R-Smads (for the TGF-β arm Smad2/3, and for the BMP arm Smad1/5/8). R-Smads form a complex with the co-Smad, Smad4, and translocate to the nucleus. TGF-β can also induce non-canonical pathways such as phospho-inositide 3′ kinase (PI3K), mitogen activated protein kinase (MEK/ERK) and small GTPase (RhoA), which will activate other transcription factors that act as co-factors to the Smad complexes. Together, the transcriptional co-factors and Smads can regulate the expression of several genes, either inducing or repressing their expression. As depicted in the text, in brain tumors the TGF-β arm of the family mostly promotes cell self-renewal and immune suppression, and the BMP arm of the family blocks self-renewal and enhances cell differentiation.

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, it 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 downregulates 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 p21^{Cip1} 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

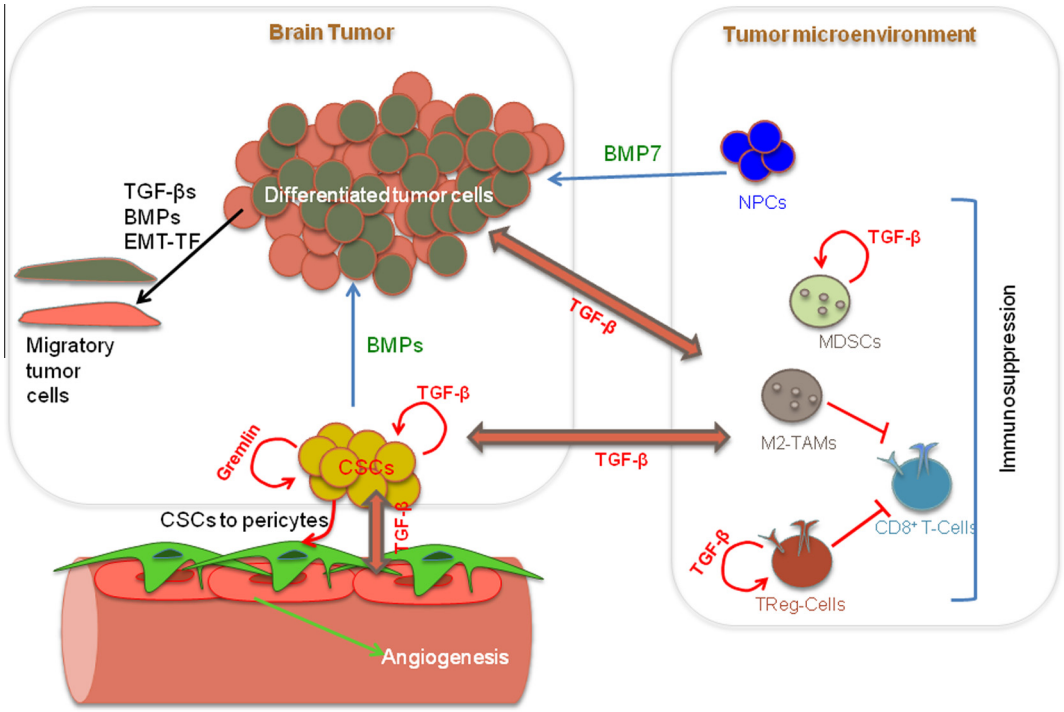

Fig. 2. Opposing actions of TGF-β and BMP signaling in glioblastoma multiforme cancer stem cells. A brain tumor made of its heterogenous differentiated cells (shown in pink and green) and their associated tumor microenvironment is schematically drawn. CSCs (yellow) produce TGF-β and Gremlin in order to maintain their self-renewal; TGF-β promotes immunosuppression in the tumor microenvironment. At the same time, the immune cells infiltrating the tumor produce TGF-β that will have an autocrine effect and enhance self-renewal of CSCs and migration of cells in the main tumor mass. TGF-β derived from CSCs induces angiogenesis in the surrounding endothelial cells. Simultaneously, endothelial cells also produce TGF-β that drives CSC to differentiate towards pericytes. Exogenous BMPs or NPC-derived BMPs act on the CSCs promoting 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.

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 micro-RNA 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 deubiquitinates 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

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 *BMPR1B* 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 BMI1 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 BMI 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 Atoh1/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 TGFBR1 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 DPIGs 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 I 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 astroglial 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-β_8 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-$\alpha_v\beta_3$ [65,66].

Glioblastomas have been divided in 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 signaling and can induce expression of Snail and Twist, whereas it binds to Snail and together repress target genes like the epithelial E-cadherin [69]. It was recently shown that TGF-β can also promote an equivalent mesenchymal differentiation in GBM and this trans-differentiation is associated with increased invasion both in vitro and in vivo and depends on the induced expression of ZEB1 [70]. TGF-β can switch cells from the proneural to a mesenchymal subtype. BMP7 has also been shown to induce GBM cell migration in a Snail-dependent manner, enhancing the expression of mesenchymal markers such as fibronectin [42]. Another factor that can promote this shift from the proneural to the mesenchymal phenotype is ionizing radiation, involving the transcriptional activity of Stat3 and CREB in Olig2$^+$ progenitor cells [71]. Whether TGF-β or EMT-TFs are also involved in the radiation-induced shift of neuroepithelial to mesenchymal cells requires further studies.

In agreement with the fact the both TGF-β and BMP can induce GBM migration through enhanced expression of EMT-TFs (Fig. 2), several reports have provided links between different EMT-TFs and glioma cell invasion. Nuclear staining of Slug and Twist1 has been observed in mesenchymal tumor areas of gliosarcomas but not in glial areas [72]. Slug has been associated with enhanced migration, stemness and tumorigenesis in glioma cells [73]. High levels of Twist1 expression are associated with the highest grade of glioma [74]. Twist1 overexpression is sufficient to increase in vitro migration and in vivo invasion; Twist1 can enhance the expression of Slug, the matrix metalloprotease MMP2 and other genes involved in invasion [75]. Snail was first reported to be necessary for GBM cell migration and proliferation in vitro [76,77] and in vivo [42]; later it was demonstrated that its expression is enhanced concomitant with a glial to mesenchymal transition after irradiation in glioma patients [78]. In vitro experiments showed that Snail expression is necessary for irradiation to induce a mesenchymal gene profile and to promote migration [78]. One mechanism for irradiation-induced Snail expression is through release of TGF-β1 by fibroblasts and macrophages resident in the tumor microenvironment [78]. Migration of GBMs can be promoted by Snail, which can repress the miR-128, which in turn represses the transcription factor SP1; the end result is enhanced expression of SP1 which cooperates with Snail in regulating the expression of pro-invasive metalloproteases [76].

ZEB2 is highly expressed in GBM tumor samples compared to normal brain tissues, and in vitro experiments show ZEB2 as being responsible for GBM cell survival and invasion, since silencing of ZEB2 is sufficient to promote apoptosis and to block cell migration [79]. ZEB1 expression correlates with poorer GBM patient survival and poor response to temozolomide (TMZ), the adjuvant chemotherapy included in standard GBM care [79]. This can be explained because ZEB1 represses the miR-200, which in turn is responsible to suppress c-MYB that upregulates the expression of O-6-methylguanine DNA methyltransferase (MGMT), the chromatin-remodeling protein responsible for TMZ resistance. Moreover, ZEB1 is highly expressed in the edges of GBM tumors, promoting GBM cell invasion by repressing miR-200, which blocks the expression of the guidance receptor ROBO1 responsible for tumor invasion [79]. Finally, the transcriptional loop ZEB1-miR-200 also regulates G-CSC stemness, controlling the expression of the stem cell factors CD133, Olig2 and Sox2 [80]. The chromatin modifying protein HMGA2 mentioned earlier as a strong promoter of EMT and metastasis [69] has also been associated with the poor prognosis of gliomas [81], which could be explained by its capacity to promote Sox2 expression and therefore a G-CSC population with tumor-initiating capacity [82].

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

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-β-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 NKG2D (natural-killer group 2, member D) in CD8$^+$ T cells and in natural killer (NK) cells; silencing of TGF-β1 and TGF-β2 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-β 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 L1CAM (L1 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 radiosensitivity 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 radioresistance was pointed out also in another study, which clarifies how inhibiting the TGF-β pathway with the TGF-βRI 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-βRI 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 new 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.

Acknowledgements

We acknowledge funding by the Ludwig Institute for Cancer Research, the Swedish Cancer Society, the Swedish Research Council and the Marie Curie Initial Training Network "IT-Liver" under the European Union FP7 program. We thank all past and present members of our group for their contributions to the scientific work emanating from our laboratory. Due to space limitations, we have been unable to include all relevant publications in our discussion. We apologize to those authors whose relevant work has not been included in this review article.

References

[1] Moustakas, A. and Heldin, C.H. (2009) The regulation of TGFβ signal transduction. Development 136, 3699–3714.
[2] Wakefield, L.M. and Hill, C.S. (2013) Beyond TGFβ: roles of other TGFβ superfamily members in cancer. Nat. Rev. Cancer 13, 328–341.
[3] Akhurst, R.J. and Hata, A. (2012) Targeting the TGFβ signalling pathway in disease. Nat. Rev. Drug Discovery 11, 790–811.
[4] Pickup, M., Novitskiy, S. and Moses, H.L. (2013) The roles of TGFβ in the tumour microenvironment. Nat. Rev. Cancer 13, 788–799.
[5] Padua, D. and Massagué, J. (2009) Roles of TGFβ in metastasis. Cell Res. 19, 89–102.
[6] Golestaneh, N. and Mishra, B. (2005) TGF-β, neuronal stem cells and glioblastoma. Oncogene 24, 5722–5730.
[7] Rich, J.N. (2003) The role of transforming growth factor-β in primary brain tumors. Front. Biosci. 8, e245–e260.
[8] Vogelstein, B., Papadopoulos, N., Velculescu, V.E., Zhou, S., Diaz, L.A. and Kinzler, K.W. (2013) Cancer genome landscapes. Science (New York, N.Y.) 339, 1546–1558.
[9] Louis, D.N., Ohgaki, H., Wiestler, O.D., Cavenee, W.K., Burger, P.C., Jouvet, A., Scheithauer, B.W. and Kleihues, P. (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 114, 97–109.
[10] Hau, P., Jachimczak, P., Schlaier, J. and Bogdahn, U. (2011) TGF-β2 signaling in high-grade gliomas. Curr. Pharm. Biotechnol. 12, 2150–2157.
[11] Puchner, M.J.A., Köppen, J.A., Zapf, S., Knabbe, C. and Westphal, M. (2002) The influence of tamoxifen on the secretion of transforming growth factor-β2 (TGF-β2) in glioblastomas: in vitro and in vivo findings. Anticancer Res. 22, 45–51.
[12] Hulshof, M.C., Sminia, P., Barten-Van Rijbroek, A.D. and Gonzalez Gonzalez, D. (2001) Prognostic value of plasma transforming growth factor-β in patients with glioblastoma multiforme. Oncol. Rep. 8, 1107–1110.
[13] Rodón, L., Gonzàlez-Juncà, A., Inda, M.d.M., Sala-Hojman, A., Martínez-Sáez, E. and Seoane, J. (2014) Active CREB1 promotes a malignant TGFβ2 autocrine loop in glioblastoma. Cancer Discov. 4, 1230–1241.
[14] Dieterich, L.C. et al. (2012) Transcriptional profiling of human glioblastoma vessels indicates a key role of VEGF-A and TGFβ2 in vascular abnormalization. J. Pathol. 228, 378–390.
[15] Shnaper, S. et al. (2009) Elevated levels of MIC-1/GDF15 in the cerebrospinal fluid of patients are associated with glioblastoma and worse outcome. International journal of cancer. J. Int. Cancer 125, 2624–2630.
[16] Bao, Z., Zhang, C., Yan, W., Liu, Y., Li, M., Zhang, W. and Jiang, T. (2013) BMP4, a strong better prognosis predictor, has a subtype preference and cell development association in gliomas. J. Transl. Med. 11, 100.
[17] Liu, C., Tian, G., Tu, Y., Fu, J., Lan, C. and Wu, N. (2009) Expression pattern and clinical prognostic relevance of bone morphogenetic protein-2 in human gliomas. Jpn. J. Clin. Oncol. 39, 625–631.
[18] Fontana, A., Bodmer, S., Frei, K., Malipiero, U. and Siepl, C. (1991) Expression of TGF-β 2 in human glioblastoma: a role in resistance to immune rejection? Ciba Found. Symp. 157, 232–238. discussion 238–41.
[19] Rich, J.N., Zhang, M., Datto, M.B., Bigner, D.D. and Wang, X.F. (1999) Transforming growth factor-β-mediated p15(INK4B) induction and growth inhibition in astrocytes is SMAD3-dependent and a pathway prominently altered in human glioma cell lines. J. Biol. Chem. 274, 35053–35058.
[20] Seoane, J., Le, H.V., Shen, L., Anderson, S.A. and Massague, J. (2004) Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. Cell 117, 211–223.
[21] Hjelmeland, A.B., Hjelmeland, M.D., Shi, Q., Hart, J.L., Bigner, D.D., Wang, X.-F., Kontos, C.D. and Rich, J.N. (2005) Loss of phosphatase and tensin homologue increases transforming growth factor β-mediated invasion with enhanced SMAD3 transcriptional activity. Cancer Res. 65, 11276–11281.
[22] Singh, S.K. et al. (2004) Identification of human brain tumour initiating cells. Nature 432, 396–401.
[23] Bao, S. et al. (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444, 756–760.
[24] Bleau, A.-M., Hambardzumyan, D., Ozawa, T., Fomchenko, E.I., Huse, J.T., Brennan, C.W. and Holland, E.C. (2009) PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell 4, 226–235.
[25] Singh, S.K., Clarke, I.D., Terasaki, M., Bonn, V.E., Hawkins, C., Squire, J. and Dirks, P.B. (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res. 63, 5821–5828.
[26] Alcantara Llaguno, S., Chen, J., Kwon, C.-H., Jackson, E.L., Li, Y., Burns, D.K., Alvarez-Buylla, A. and Parada, L.F. (2009) Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. Cancer Cell 15, 45–56.
[27] Chen, J., Li, Y., Yu, T.-S., McKay, R.M., Burns, D.K., Kernie, S.G. and Parada, L.F. (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488, 522–526.
[28] Holland, E.C., Celestino, J., Dai, C., Schaefer, L., Sawaya, R.E. and Fuller, G.N. (2000) Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat. Genet. 25, 55–57.
[29] Alcantara Llaguno, S.R., Chen, Y., McKay, R.M. and Parada, L.F. (2011) Stem cells in brain tumor development. Curr. Top. Dev. Biol. 94, 15–44.
[30] Bruna, A. et al. (2007) High TGFβ-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. Cancer Cell 11, 147–160.
[31] Ikushima, H., Komuro, A., Isogaya, K., Shinozaki, M., Hellman, U., Miyazawa, K. and Miyazono, K. (2008) An Id-like molecule, HHM, is a synexpression group-restricted regulator of TGF-β signalling. EMBO J. 27, 2955–2965.
[32] Lee, C.-C. et al. (2010) Nodal promotes growth and invasion in human gliomas. Oncogene 29, 3110–3123.
[33] Sun, J., Liu, S.-Z., Lin, Y., Cao, X.-P. and Liu, J.-M. (2014) TGF-β promotes glioma cell growth via activating Nodal expression through Smad and ERK1/2 pathways. Biochem. Biophys. Res. Commun. 443, 1066–1072.
[34] Kingsley-Kallesen, M., Luster, T.A. and Rizzino, A. (2001) Transcriptional regulation of the transforming growth factor-β2 gene in glioblastoma cells. In Vitro Cell. Dev. Biol. Anim. 37, 684–690.
[35] Song, L. et al. (2012) TGF-β induces miR-182 to sustain NF-κB activation in glioma subsets. J. Clin. Investig. 122, 3563–3578.
[36] Ikushima, H., Todo, T., Ino, Y., Takahashi, M., Miyazawa, K. and Miyazono, K. (2009) Autocrine TGF-β signaling maintains tumorigenicity of glioma-initiating cells through Sry-related HMG-box factors. Cell Stem Cell 5, 504–514.
[37] Peñuelas, S. et al. (2009) TGF-β increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. Cancer Cell 15, 315–327.
[38] Anido, J. et al. (2010) TGF-β receptor inhibitors target the CD44(high)/Id1(high) glioma-initiating cell population in human glioblastoma. Cancer Cell 18, 655–668.
[39] Eichhorn, P.J.A. et al. (2012) USP15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. Nat. Med. 18, 429–435.

[40] Piccirillo, S.G.M. et al. (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444, 761–765.
[41] Tate, C.M. et al. (2012) A BMP7 variant inhibits the tumorigenic potential of glioblastoma stem-like cells. Cell Death Differ. 19, 1644–1654.
[42] Savary, K. et al. (2013) Snail depletes the tumorigenic potential of glioblastoma. Oncogene 32, 5409–5420.
[43] Lee, J. et al. (2008) Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell 13, 69–80.
[44] Gargiulo, G., Cesaroni, M., Serresi, M., de Vries, N., Hulsman, D., Bruggeman, S.W., Lancini, C. and van Lohuizen, M. (2013) In vivo RNAi screen for BMI1 targets identifies TGF-β/BMP-ER stress pathways as key regulators of neural- and malignant glioma-stem cell homeostasis. Cancer Cell 23, 660–676.
[45] Yan, K. et al. (2014) Glioma cancer stem cells secrete Gremlin1 to promote their maintenance within the tumor hierarchy. Genes Dev. 28, 1085–1100.
[46] Guo, M. et al. (2014) MiR-656 inhibits glioma tumorigenesis through repression of BMPR1A. Carcinogenesis 35, 1698–1706.
[47] Hatten, M.E. and Roussel, M.F. (2011) Development and cancer of the cerebellum. Trends Neurosci. 34, 134–142.
[48] Alvarez-Rodriguez, R., Barzi, M., Berenguer, J. and Pons, S. (2007) Bone morphogenetic protein 2 opposes Shh-mediated proliferation in cerebellar granule cells through a TIEG-1-based regulation of Nmyc. J. Biol. Chem. 282, 37170–37180.
[49] Zhao, H., Ayrault, O., Zindy, F., Kim, J.-H. and Roussel, M.F. (2008) Post-transcriptional down-regulation of Atoh1/Math1 by bone morphogenic proteins suppresses medulloblastoma development. Genes Dev. 22, 722–727.
[50] Hallahan, A.R. et al. (2003) BMP-2 mediates retinoid-induced apoptosis in medulloblastoma cells through a paracrine effect. Nat. Med. 9, 1033–1038.
[51] Fiaschetti, G. et al. (2011) Bone morphogenetic protein-7 is a MYC target with prosurvival functions in childhood medulloblastoma. Oncogene 30, 2823–2835.
[52] Northcott, P.A. et al. (2012) Subgroup-specific structural variation across 1000 medulloblastoma genomes. Nature 488, 49–56.
[53] Aref, D. et al. (2013) Canonical TGF-β pathway activity is a predictor of SHH-driven medulloblastoma survival and delineates putative precursors in cerebellar development. Brain Pathol. 23, 178–191.
[54] Jones, C. and Baker, S.J. (2014) Unique genetic and epigenetic mechanisms driving paediatric diffuse high-grade glioma. Nat. Rev. Cancer 14.
[55] Buczkowicz, P. et al. (2014) Genomic analysis of diffuse intrinsic pontine gliomas identifies three molecular subgroups and recurrent activating ACVR1 mutations. Nat. Genet. 46, 451–456.
[56] Fontebasso, A.M. et al. (2014) Recurrent somatic mutations in ACVR1 in pediatric midline high-grade astrocytoma. Nat. Genet. 46, 462–466.
[57] Taylor, K.R. et al. (2014) Recurrent activating ACVR1 mutations in diffuse intrinsic pontine glioma. Nat. Genet. 46, 457–461.
[58] Wu, G. et al. (2014) The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat. Genet. 46, 444–450.
[59] Pistollato, F. et al. (2009) Hypoxia and HIF1α repress the differentiative effects of BMPs in high-grade glioma. Stem Cells 27, 7–17.
[60] Srikanth, M., Kim, J., Das, S. and Kessler, J.A. (2014) BMP signaling induces astrocytic differentiation of clinically derived oligodendroglioma propagating cells. Mol. Cancer Res. 12, 283–294.
[61] Kwiatkowska, A. and Symons, M. (2013) Signaling determinants of glioma cell invasion. Adv. Exp. Med. Biol. 986, 121–141.
[62] Wesolowska, A. et al. (2008) Microglia-derived TGF-β as an important regulator of glioblastoma invasion – an inhibition of TGF-β-dependent effects by shRNA against human TGF-β type II receptor. Oncogene 27, 918–930.
[63] Mobley, A.K., Tchaicha, J.H., Shin, J., Hossain, M.G. and McCarty, J.H. (2009) β8 integrin regulates neurogenesis and neurovascular homeostasis in the adult brain. J. Cell Sci. 122, 1842–1851.
[64] Tchaicha, J.H., Reyes, S.B., Shin, J., Hossain, M.G., Lang, F.F. and McCarty, J.H. (2011) Glioblastoma angiogenesis and tumor cell invasiveness are differentially regulated by β8 integrin. Cancer Res. 71, 6371–6381.
[65] Baumann, F. et al. (2009) Lactate promotes glioma migration by TGF-β2-dependent regulation of matrix metalloproteinase-2. Neuro-oncology 11, 368–380.
[66] Wick, W., Platten, M. and Weller, M. (2001) Glioma cell invasion: regulation of metalloproteinase activity by TGF-β. J. Neurooncol. 53, 177–185.
[67] Verhaak, R.G.W. et al. (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17, 98–110.
[68] Phillips, H.S. et al. (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9, 157–173.
[69] Tan, E.-J., Olsson, A.-K. and Moustakas, A. (2014) Reprogramming during epithelial to mesenchymal transition under the control of TGFβ. Cell Adhes. Migration.
[70] Joseph, J.V. et al. (2014) TGF-β is an inducer of ZEB1-dependent mesenchymal transdifferentiation in glioblastoma that is associated with tumor invasion. Cell Death Dis. 5, e1443.
[71] Halliday, J., Helmy, K., Pattwell, S.S., Pitter, K.L., LaPlant, Q., Ozawa, T. and Holland, E.C. (2014) In vivo radiation response of proneural glioma characterized by protective p53 transcriptional program and proneural–mesenchymal shift. Proc. Natl. Acad. Sci. U.S.A. 111, 5248–5253.
[72] Nagaishi, M., Paulus, W., Brokinkel, B., Vital, A., Tanaka, Y., Nakazato, Y., Giangaspero, F. and Ohgaki, H. (2012) Transcriptional factors for epithelial–mesenchymal transition are associated with mesenchymal differentiation in gliosarcoma. Brain Pathol. 22, 670–676.
[73] Xia, H. et al. (2012) Loss of brain-enriched miR-124 microRNA enhances stem-like traits and invasiveness of glioma cells. J. Biol. Chem. 287, 9962–9971.
[74] Elias, M.C. et al. (2005) TWIST is expressed in human gliomas and promotes invasion. Neoplasia (New York, N.Y.) 7, 824–837.
[75] Mikheeva, S.A. et al. (2010) TWIST1 promotes invasion through mesenchymal change in human glioblastoma. Mol. Cancer 9, 194.
[76] Dong, Q. et al. (2014) An axis involving SNAI1, microRNA-128 and SP1 modulates glioma progression. PLoS One 9, e98651.
[77] Han, S.-P. et al. (2011) SNAI1 is involved in the proliferation and migration of glioblastoma cells. Cell. Mol. Neurobiol. 31, 489–496.
[78] Mahabir, R. et al. (2014) Sustained elevation of Snail promotes glial-mesenchymal transition after irradiation in malignant glioma. Neuro-oncology 16, 671–685.
[79] Qi, S. et al. (2012) ZEB2 mediates multiple pathways regulating cell proliferation, migration, invasion, and apoptosis in glioma. PLoS One 7, e38842.
[80] Siebzehnrubl, F.A. et al. (2013) The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance. EMBO Mol. Med. 5, 1196–1212.
[81] Liu, B. et al. (2014) Expression of high-mobility group AT-hook protein 2 and its prognostic significance in malignant gliomas. Hum. Pathol. 45, 1752–1758.
[82] Chiou, G.-Y. et al. (2013) Epigenetic regulation of the miR142-3p/interleukin-6 circuit in glioblastoma. Mol. Cell 52, 693–706.
[83] Calabrese, C. et al. (2007) A perivascular niche for brain tumor stem cells. Cancer Cell 11, 69–82.
[84] Bao, S. et al. (2006) Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res. 66, 7843–7848.
[85] Cheng, L. et al. (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. Cell 153, 139–152.
[86] Pen, A., Moreno, M.J., Durocher, Y., Deb-Rinker, P. and Stanimirovic, D.B. (2008) Glioblastoma-secreted factors induce IGFBP7 and angiogenesis by modulating Smad-2-dependent TGF-β signaling. Oncogene 27, 6834–6844.
[87] Schulte, J.D. et al. (2013) Cadherin-11 regulates motility in normal cortical neural precursors and glioblastoma. PLoS One 8, e70962.
[88] Wurdinger, T., Deumelandt, K., van der Vliet, H.J., Wesseling, P. and de Gruijl, T.D. (2014) Mechanisms of intimate and long-distance cross-talk between glioma and myeloid cells: how to break a vicious cycle. Biochim. Biophys. Acta 1846, 560–575.
[89] Suzumura, A., Sawada, M., Yamamoto, H. and Marunouchi, T. (1993) Transforming growth factor-β suppresses activation and proliferation of microglia in vitro. J. Immunol. 151, 2150–2158.
[90] Zhai, H., Heppner, F.L. and Tsirka, S.E. (2011) Microglia/macrophages promote glioma progression. Glia 59, 472–485.
[91] Markovic, D.S., Glass, R., Synowitz, M., Rooijen, N.v. and Kettenmann, H. (2005) Microglia stimulate the invasiveness of glioma cells by increasing the activity of metalloprotease-2. J. Neuropathol. Exp. Neurol. 64, 754–762.
[92] Ye, X.-Z. et al. (2012) Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-β1 signaling pathway. J. Immunol. 189, 444–453.
[93] Umemura, N. et al. (2008) Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. J. Leukoc. Biol. 83, 1136–1144.
[94] Ueda, R. et al. (2009) Systemic inhibition of transforming growth factor-β in glioma-bearing mice improves the therapeutic efficacy of glioma-associated antigen peptide vaccines. Clin. Cancer Res. 15, 6551–6559.
[95] Liang, H., Yi, L., Wang, X., Zhou, C. and Xu, L. (2014) Interleukin-17 facilitates the immune suppressor capacity of high-grade glioma-derived CD4 (+) CD25 (+) Foxp3 (+) T cells via releasing transforming growth factor β. Scand. J. Immunol. 80, 144–150.
[96] Crane, C.A., Han, S.J., Barry, J.J., Ahn, B.J., Lanier, L.L. and Parsa, A.T. (2010) TGF-β downregulates the activating receptor NKG2D on NK cells and $CD8^+$ T cells in glioma patients. Neuro-oncology 12, 7–13.
[97] Friese, M.A., Wischhusen, J., Wick, W., Weiler, M., Eisele, G., Steinle, A. and Weller, M. (2004) RNA interference targeting transforming growth factor-β enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo. Cancer Res. 64, 7596–7603.
[98] Lohr, J. et al. (2011) Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-β. Clin. Cancer Res. J. Am. Assoc. Cancer Res. 17, 4296–4308.
[99] Gate, D. et al. (2014) T-cell TGF-β signaling abrogation restricts medulloblastoma progression. Proc. Natl. Acad. Sci. U.S.A. 111, E3458–E3466.
[100] Glass, R. et al. (2005) Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. J. Neurosci. J. Soc. Neurosci. 25, 2637–2646.
[101] Chirasani, S.R. et al. (2010) Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain: J. Neurol. 133, 1961–1972.
[102] Pistollato, F. et al. (2009) Molecular mechanisms of HIF-1α modulation induced by oxygen tension and BMP2 in glioblastoma derived cells. PLoS One 4, e6206.

[103] Cheng, L., Bao, S. and Rich, J.N. (2010) Potential therapeutic implications of cancer stem cells in glioblastoma. Biochem. Pharmacol. 80, 654–665.
[104] Persano, L. et al. (2012) BMP2 sensitizes glioblastoma stem-like cells to temozolomide by affecting HIF-1α stability and MGMT expression. Cell Death Dis. 3, e412.
[105] Duggal, R., Geissinger, U., Zhang, Q., Aguilar, J., Chen, N.G., Binda, E., Vescovi, A.L. and Szalay, A.A. (2013) Vaccinia virus expressing bone morphogenetic protein-4 in novel glioblastoma orthotopic models facilitates enhanced tumor regression and long-term survival. J. Transl. Med. 11, 155.
[106] Kerr, G., Sheldon, H., Chaikuad, A., Alfano, I., von Delft, F., Bullock, A.N. and Harris, A.L. (2015) A small molecule targeting ALK1 prevents Notch cooperativity and inhibits functional angiogenesis. Angiogenesis.
[107] Reguera-Nuñez, E., Roca, C., Hardy, E., de la Fuente, M., Csaba, N. and Garcia-Fuentes, M. (2014) Implantable controlled release devices for BMP-7 delivery and suppression of glioblastoma initiating cells. Biomaterials 35, 2859–2867.
[108] P. Hau, et al., Inhibition of TGF-β2 with AP 12009 in Recurrent Malignant Gliomas: From Preclinical to Phase I/II Studies, 2007.
[109] Zhang, M. et al. (2011) Blockade of TGF-β signaling by the TGFβR-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. Cancer Res. 71, 7155–7167.
[110] Hardee, M.E., Marciscano, A.E., Medina-Ramirez, C.M., Zagzag, D., Narayana, A., Lonning, S.M. and Barcellos-Hoff, M.H. (2012) Resistance of glioblastoma-initiating cells to radiation mediated by the tumor microenvironment can be abolished by inhibiting transforming growth factor-β. Cancer Res. 72, 4119–4129.
[111] Rodon, J. et al. (2015) First-in-human dose study of the novel transforming growth factor-β receptor I kinase inhibitor LY2157299 monohydrate in patients with advanced cancer and glioma. Clin. Cancer Res. J. Am. Assoc. Cancer Res. 21, 553–560.
[112] Pham, K., Luo, D., Siemann, D.W., Law, B.K., Reynolds, B.A., Hothi, P., Foltz, G. and Harrison, J.K. (2015) VEGFR inhibitors upregulate CXCR4 in VEGF receptor-expressing glioblastoma in a TGFβR signaling-dependent manner. Cancer Lett. 360, 60–67.