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REVIEW **Open Access**

TGF-β links glycolysis and immunosuppression in glioblastoma

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Summary. Glioblastoma (GBM) is the most common and aggressive brain tumor in adults, characterized by diffuse infiltration, dysplasia, and resistance to therapy. Metabolic remodeling and immunosuppression are typical events which contribute to GBM progression, but the molecular link between these two events remains largely undetermined. Studies have shown that high levels of transforming growth factor-β (TGF-β) and its receptors are associated with glioma malignancy and a poor prognosis. TGF-β plays an important role in cell metabolism and immunity. During tumorigenesis, TGF-β induces a shift in cell metabolism from oxidative phosphorylation to aerobic glycolysis, providing a favorable environment for tumor growth. Locally, TGF-β creates an immunosuppressive microenvironment and promotes the malignant phenotype of GBM. In this review, we aim to link GBM aerobic glycolysis and immunosuppression through TGF-β to provide new ideas for the study of GBM.

Key words: TGF-β, Immunosuppression, GBM, Glycolysis

Introduction

Glioblastoma (GBM) is the most common primary malignant tumor of the central nervous system. Despite multidisciplinary treatment, relapse is inevitable. One of the main factors is the interaction of tumor cells with the complex microenvironment, leading to high proliferation, invasion and treatment escape (Bonavia et al., 2011; Nakada et al., 2011). In recent decades, metabolic remodeling has gradually become a hot topic in cancer research, among which the Warburg effect is a frequent theme. The Warburg theory of cancer or the "Warburg hypothesis" holds that in the presence of glucose, glycolysis (which is inhibited in normal cells) is activated in cancers, even with sufficient oxygen. This process provides lactate as the major energy source for tumor cells (Warburg et al., 1927). Transforming growth factor-β (TGF-β), which contains three isoforms including TGF-β1, TGF-β2 and TGF-β3, is a member of the superfamily of growth and differentiation factors. TGF-β is widely implicated in human diseases, including carcinogenesis, development, and cancer recurrence. The activation of TGF-β is mainly regulated by the conversion of latent TGF-β to active TGF-β. Once activated, it binds and activates the TGF-β receptor, triggering phosphorylation of the intracellular effectors, SMADs. Given that TGF-β/TGF-β receptor dysregulation has been implicated during different stages of cancer development and that they play pivotal roles in tumor progression, this pathway is an attractive target for cancer therapy (Smith et al., 2012; Ahmadi et al., 2019). Among other roles, TGF-β exerts immunosuppressive regulation in cancers. Particularly, in invasive GBM, TGF-β is overexpressed and impinges on the antitumor immune response (Nana et al., 2015). In addition to immunosuppressive effects, TGF-β has been associated with increased glycolysis in cancers (Lee et al., 2015; Zhang et al., 2015; Rodríguez-García et al., 2017; Hu et al., 2018; Wang et al., 2018). Gene set enrichment analysis (GSEA) has also confirmed that TGF-β is closely related to the inflammation and glycolysis pathways in GBM (Fig. 1). The influence of glycolysis on tumor immune regulation has become increasingly recognized as an important factor of tumor growth and progression (Kesarwani et al., 2017; Li et al., 2018; Gupta and Dwarakanath, 2020; Urata et al., 2020). This review provides an updated understanding of TGF-β on glycolysis and immunosuppression in GBM, discussing the link between these two biological processes via TGF-β signaling.

The structure and function of TGF-β

TGF-β in mammals includes three subtypes, TGF-β1, 2 and 3, which are synthesized as precursors (pro-TGF-β), including an N-terminal latency-related peptide (LAP) and a C-terminal growth factor (GF) (active

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subunit). TGF-β subtypes are highly homologous, with the active subunit region showing 71-79% amino acid sequence homology, while the LAP region shows slightly less (about 36-51%) (Travis and Sheppard, 2014). In the endoplasmic reticulum, pro-TGF-β is dimerized, folded, and disulfide bound with late TGF-β-binding protein (LTBP) to form ternary complex. LTBP is necessary for the folding, secretion and targeting of TGF-β to extracellular structures (Govinden and Bhoda, 2003). When the complex is transported to the Golgi apparatus, the polypeptide connection between LAP and GF is cleaved by furin. However, GF is still strongly bound to LAP in a noncovalent manner, causing storage of TGF-β in a latent state in the extracellular environment. After secretion, LTBP interacts with other components of the extracellular matrix, releasing active TGF-β (Robertson and Rifkin, 2016; Zhao et al., 2018).

Three protein subtypes of TGF-β are abundantly observed during development, displaying overlapping but unique spatial-temporal expression patterns (Thompson et al., 1989; Gatherer et al., 1990; Pelton et al., 1990). TGF-β regulates a variety of common physiological and pathological processes, including angiogenesis, cell proliferation, wound healing, immunity, and cancer. Each subtype has unique effects depending on cell type, differentiation status, growth conditions, and the presence of other growth factors. For example, TGF-β1 plays a bidirectional role in angiogenesis *in vitro*. High concentrations of TGF-β1 can inhibit endothelial cell invasion and capillary lumen formation, while low concentrations can enhance it (Pepper et al., 1993). *In vivo*, the response of endothelial cells to TGF-β1 may be regulated by changes in cell phenotype and the composition of the surrounding matrix during angiogenesis (Sutton et al., 1991). The antiproliferative response of TGF-β is observed in many cell types, and the mechanism for growth inhibition may include the down-regulation of proliferative protein c-myc and up-regulation of cell cycle inhibitory proteins $p15^{INK4B}$ and $p21^{CIP1}$ (Alexandrow and Moses, 1995;

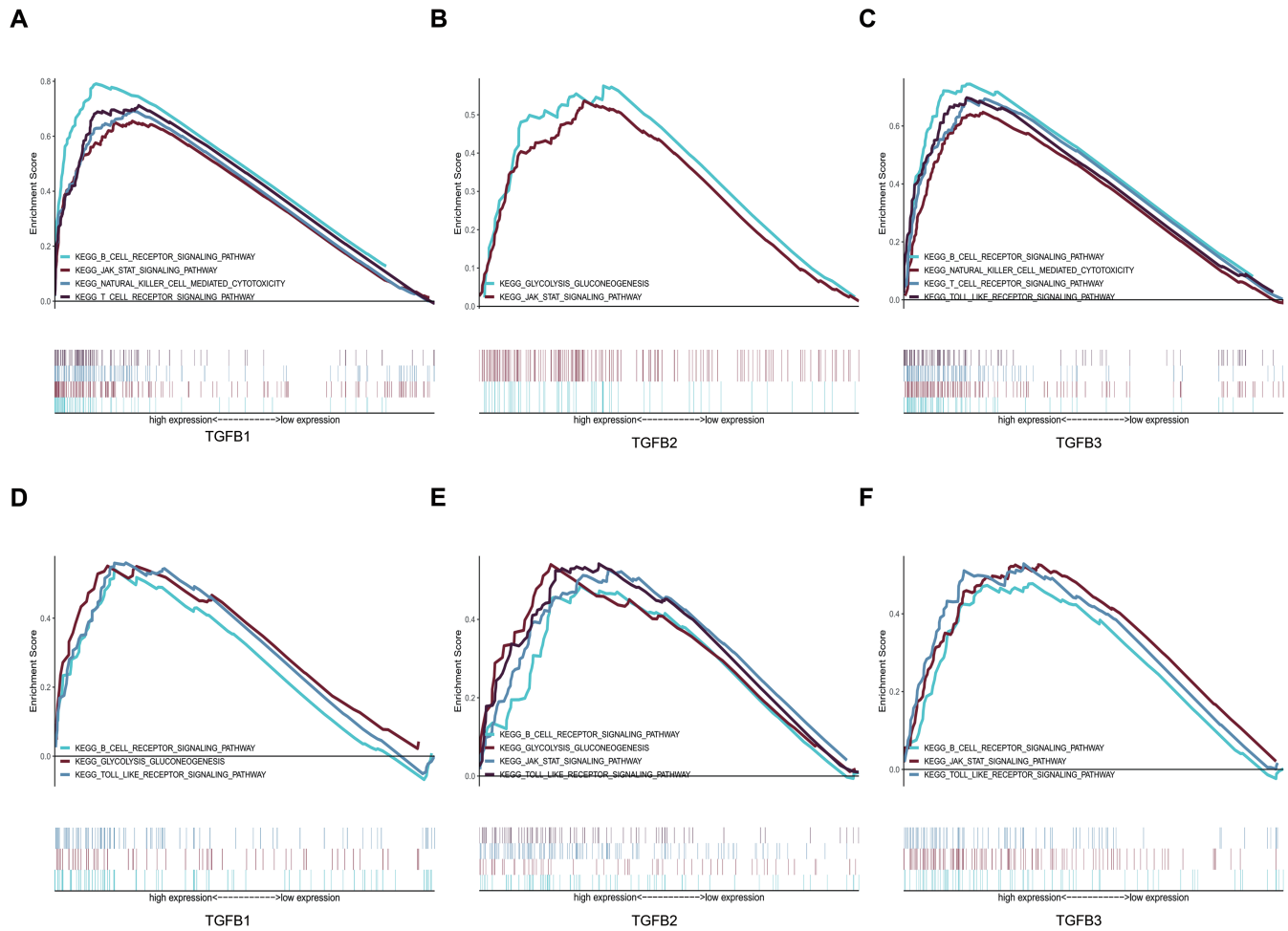

Fig. 1. TGF-β expression is associated with glycolysis and immunosuppression. **A-C.** GSEA analysis of high expression of TGF-β (TGFB1, TGFB2 and TGFB3) in The Cancer Genome Atlas (TCGA) database. **D-F.** GSEA analysis of high expression of TGF-β (TGFB1, TGFB2 and TGFB3) in The Chinese Glioma Genome Atlas (CGGA) database.

Ravitz and Wenner, 1997; Warner et al., 1999). Under certain conditions it can also promote cell proliferation such as in the case of mesenchymal stem cells (Jian et al., 2006). Various TGF-β isoforms may have different effects on wound healing. TGF-β1 may mediate fibrosis of adult wounds, while TGF-β3 may promote scarless healing of the fetus and reduce scarring in adults (Lichtman et al., 2016). On the immune front, TGF-β1 deficient mice show an autoimmune disease demonstrating that TGF-β1 contributes to immune homeostasis maintenance (Kulkarni et al., 1993). TGF-β2 is involved in immunosuppression, including the induction of glioblastoma-derived T cell inhibitory factor (G-TsF). Finally, the isoform TGF-β3 has demonstrated both pro-inflammatory and anti-inflammatory effects (de Martin et al., 1987; Okamura et al., 2015).

The role of TGF-β in tumorigenesis is complex and contradictory. It plays an inhibitory role in normal and early tumors. As tumors continue to develop, the tumor inhibitory function of TGF-β is circumvented, making TGF-β a pro-cancer factor (Seoane and Gomis, 2017). The dichotomy of TGF-β in tumors is called the "TGF-β paradox". Loss of tumor response to TGF-β inhibition may be due to a mutation in the TGF-β signaling cascade or a cellular inhibition program that selectively weakens TGF-β (Seoane and Gomis, 2017).

Smad-dependent and Smad-independent TGF-β signaling pathways

Typical TGF-β signaling involves a series of signaling processes mediated by by ligand binding to receptors (Fig. 2). There are three types of transmembrane TGF-β receptors: type I receptor (TβRI), type II receptor (TβRII) and type III receptor (TβRIII) (Massagué, 1990). Upon binding to TGF-β, TβRII activates and recruits TβRI to form a receptor dimer via phosphorylation of the serine and threonine residues. Subsequently, the activated TβRI induces the phosphorylation of downstream Smad proteins (Hata and Chen, 2016). There are 8 types of Smad proteins, 3 of which are involved in TGFβ signaling, the receptor-regulated (R), the common (Co) and the inhibitory Smads (I) (Hata and Chen, 2016). TβRI phosphorylates two serine residues in the carboxy-terminal Ser-Ser-X-Ser (SSXS) sequence motif of R-Smad to activate R-Smad (Smad2/3). Activated R-Smad and Co-Smad (Smad4) then form a hetero-oligomeric complex which translocates into the nucleus and regulates the expression of target genes (Hata and Chen, 2016). I-Smad (Smad6/7) inhibits TGF-β signaling by mediating TβRI degradation via the ubiquitin proteasome system, interfering with the R-Smad-Smad4 complex by competitively interacting with R-Smads, consequently

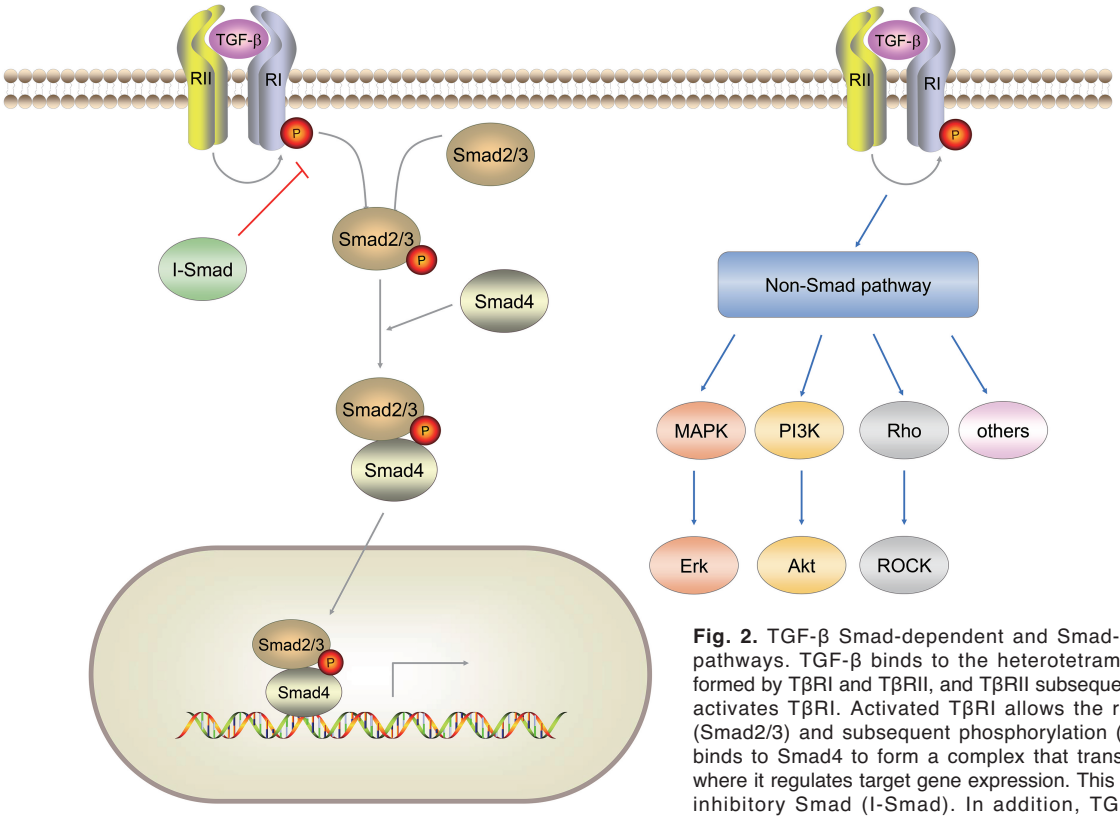

Fig. 2. TGF-β Smad-dependent and Smad-independent signaling pathways. TGF-β binds to the heterotetrameric receptor complex formed by TβRI and TβRII, and TβRII subsequently phosphorylates and activates TβRI. Activated TβRI allows the recruitment of R-Smad (Smad2/3) and subsequent phosphorylation (pSmad2/3). pSmad2/3 binds to Smad4 to form a complex that translocates to the nucleus where it regulates target gene expression. This pathway is regulated by inhibitory Smad (I-Smad). In addition, TGF-β can regulate cell processes by activating Smad-independent signaling pathways such as MAPK, PI3K, and Rho.

inhibiting the transcriptional activity of the R-Smad–Smad4 complex. Inhibitory smads may also directly bind target DNA (Miyazawa and Miyazono, 2017). In addition to the typical Smad pathway, there is crosstalk between TGF-β/TGF-β receptor and non-Smad signaling pathways, such as mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K/AKT), and others (Wnt, Notch, IL, INF-γ) (Guo and Wang, 2009; Zhang, 2017).

TGF-β and GBM

GBM is a malignant tumor characterized by extreme genetic instability. Genomic differences and protein expression differences in tumor cells are particularly common. The expression of TGF-β in GBM patients has been characterized by The Cancer Genome Atlas (TCGA). Two isoforms (TGF-β1 and TGF-β2) are significantly increased compared with normal tissues, and TGF-β3 also demonstrates a tendency towards increase (Fig. 3). Disordered TGF-β is a carcinogenic factor for GBM, supporting tumor growth, promoting invasion and angiogenesis, and producing an immunosuppressive microenvironment (Joseph et al., 2013; Caja et al., 2015). Epithelial-mesenchymal transition (EMT) is the core mechanism that confers invasiveness to GBM cells. TGF-β is a strong promoter of EMT and induces EMT-transcription factors (EMT-TFs) through Smad or non-Smad pathways, including SNAIL1, SNAIL2, ZEB1/2 and TWIST (Hao et al., 2019). Moreover, the migration associated genes MMP2 and ADAM17 are also targets of TGF-β (Wick et al., 2001; Lu et al., 2011). Angiogenesis is a hallmark of malignant gliomas. As an angiogenic factor, TGF-β can directly induce angiogenesis, or indirectly induce angiogenesis by increasing the expression and activity of certain angiogenic proteins and cytokines (such as VEGF, PDGF and integrity) (Roberts et al., 1986; Jennings et al., 1997; Roth et al., 2013; Krishnan et al., 2015). Additionally, one recent study (Rodríguez-García et al., 2017), as well as the GSEA analysis from the TCGA and CGGA GBM data sets, gives evidence to connect TGF-β to glycolysis in GBM (Fig. 1).

TGF-β has been connected to the establishing immunological tolerance of GBM. Early studies have identified the immunosuppressive effects of TGF-β2 in GBM, which are reflected by its protein name, synonymous with glioblastoma-derived T cell inhibitory factor (G-TsF) (de Martin et al., 1987; Kuppner et al., 1988; Siepl et al., 1988). Subsequently, the effects of TGF-β, especially TGF-β1 and β2, in reducing immune-surveillance of glioma development are evident in the inhibition of lymphocyte activation of killer (LAK) cell activity, suppression of tumor-infiltrating lymphocyte (TIL's) cytotoxic response, reduction of tumor surface antigen expression and alteration of immune cell phenotypes (Kuppner et al., 1988, 1989; Zuber et al., 1988; Eisele et al., 2006; Crane et al., 2010; Close et al., 2020). The pathogenic relevance of TGF-β3 in GBM largely remains to be elucidated. What is known is that inhibition of TGF-β3 expression blocks the phosphorylation of downstream SMAD2 and SMAD1/5 without affecting TGF-β1 or TGF-β2. Interestingly, the loss of TGF-β3 attenuates SMAD signal transduction induced by TGF-β1 or TGF-β2, suggesting that TGF-β3 may be a gatekeeper of TGF-β/Smads signaling (Seystahl et al., 2017).

Although the role of TGF-β in promoting GBM progression has been well established, the effect of TGF-β on GBM remains controversial, consistent with the "TGF-β paradox". Studies on different glioma cell lines have revealed that TGF-β can positively or negatively regulate the proliferation of tumors, which may be dependent on the expression levels of TGF-β receptor and Smad (Piek et al., 1999). Glioma cells isolated from three patients were treated with serum, one of which

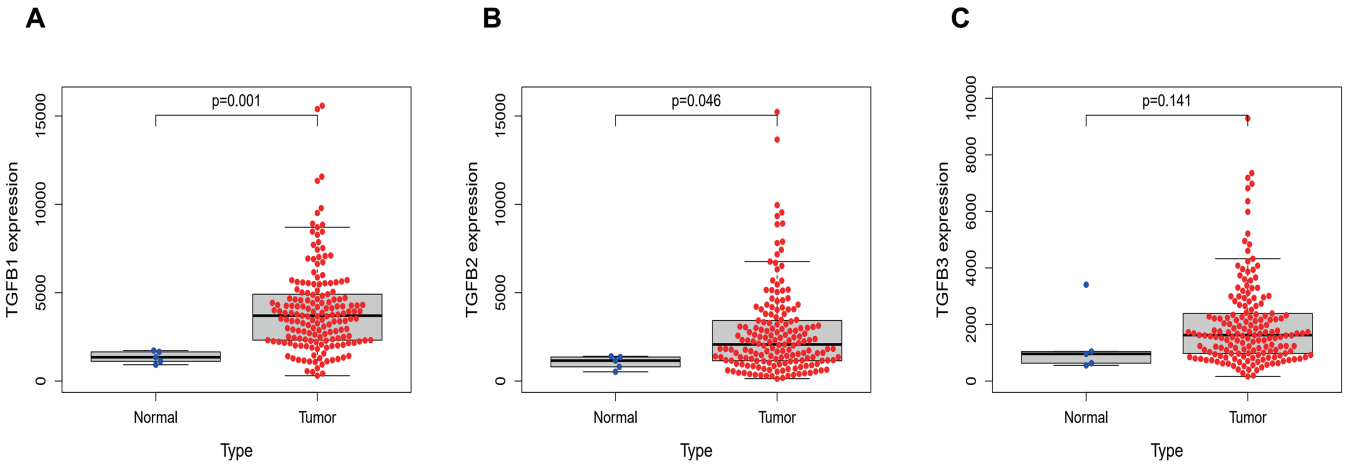

Fig. 3. TGF-β is highly expressed in GBM samples. **A.** TGFB1 expression in normal group and GBM group in TCGA database. **B.** TGFB2 expression in normal group and GBM group in TCGA database. **C.** TGFB3 expression in normal group and GBM group in TCGA database.

showed TGF-β-induced senescence while the cells of the other two patients did not respond, possibly because the cells lacked the expression of a protein that activates potential TGF-β (Kumar et al., 2017). In murine glioma models, the combined treatment of TGF-β receptor inhibitor and vascular endothelial growth factor antibody had different effects on different models. The model for prolonging the survival time was related to early pSMAD2 inhibition, long-term vascular inhibition and delayed accumulation of microglia/macrophages, while the ineffective model was related to high hypoxia level (Mangani et al., 2016). Whether TGF-β can promote or inhibit proliferation is partly due to intracellular epigenetic changes such as methylation of the PDGF-B gene (Bruna et al., 2007). Studies have also shown that the imbalance between Smad and MAPK pathways is responsible for TGF-β carcinogenesis (Nickl-Jockschat et al., 2007). Furthermore, the interacting Smad partners are a factor that cannot be ignored (Xu et al., 2015). As mentioned above, TGF-β induces the expression of the cell cycle inhibitory protein $p21^{Cip1}$. High levels of the transcription factor FoxG1 in glioblastoma cells destroy the Smad-FoxO3 complex, which is key to turning on the growth inhibitory gene $p21^{Cip1}$ (Seoane et al., 2004). As a result, the growth inhibition of TGF-β is suppressed.

TGF-β and glioma stem cells

Glioma stem cell (GSC) subpopulations have been identified in gliomas and likely play key roles in tumor maintenance, recurrence, and therapeutic resistance (Singh et al., 2004; Bao et al., 2006). GSCs are characterized by the ability to express the common neural stem cell markers including CD133, CD15, Sox2 and Nestin (Ludwig and Kornblum, 2017). TGF-β plays a central role in various aspects of nervous system development and function (Golestaneh and Mishra, 2005). On this basis, Ikushima et al. found that TGF-β signaling can induce the stemness gene Sox2, and this induction is mediated by Sox4, a direct TGF-β target gene (Ikushima et al., 2009). In the same year, Peñuelas et al. identified the molecular mechanism of TGF-β regulating GSCs' self-renewal, mainly, the Smad-dependent LIF-JAK-STAT pathway (Peñuelas et al., 2009). Recent studies have also shown that lncRNA is involved in the TGF-β-induced self-renewal of GSCs (Tang et al., 2019). Therefore, TGF-β signaling is essential for the self-renewal and tumorigenicity of GSCs. On the other hand, the infiltration of glioma-related microglia/macrophages (GAMs) is correlated with the presence of GSCs, and GAMs produce high levels of TGF-β1 in a feedback loop, indicating that TGF-β is a key factor for the dynamic crosstalk between GSCs and immune cells (Ye et al., 2012). TGF-β1 promotes the expression of MMP-9 to increase GSC invasion potential (Ye et al., 2012). In turn, GSCs lead to increased expression of M2 markers in macrophages (Nusblat et al., 2017). Regulatory T cells (Tregs) induce GSC enrichment by secretion of TGF-β and promote GSC quality by TGF-β-NF-κB-IL6-STAT3 signaling (Liu et al., 2021a). Therefore, an ideal solution for overcoming radiation resistance could include the suppression of GSC pools via TGF-β pathway inhibition.

TGF-β and glycolysis

The earilest studies of the relationship between TGF-β and glycolysis were reported in the 1980s, indicating that TGF-β increases the uptake of glucose and stimulates glycolysis (Boerner et al., 1985; Taylor et al., 1989). Subsequently, the induction of TGF-β1 by glycolysis in tumor cells has been demonstrated to be associated with several pathways. For example, Lee et al. reported that TGF-β/Wnt signaling inhibits the expression of cytochrome C oxidase (COX) in a Dlx-2/Snail-dependent manner, resulting in a decline of mitochondrial respiration of cancer cells and an increase in glycolysis (Lee et al., 2015). TGF-β1 also induces cancer-related fibroblast (CAF) switch from oxidative phosphorylation to glycolysis via downregulation of isocitrate dehydrogenase 3α (IDH3α) and the stabilization of HIF-1α (Zhang et al., 2015). In addition to reducing oxidative phosphorylation, TGF-β can increase tumor glycolysis by affecting transporters and essential enzymes during glycolysis. For example, TGF-β stimulation has been shown to increase glucose transporter (GLUT) levels in some cancers (Li et al., 2010; Rodríguez-García et al., 2017; Osumi et al., 2020). This allows cancer cells to take up glucose more quickly for use. The primary restriction enzyme hexokinase 2 (HK2) of glycolysis mediates the conversion of glucose to glucose 6-phosphate and prevents glucose from leaving the cell. In liver cancer cells, TGF-β1 upregulates HK2, providing an important step in increasing glycolytic flux (Hu et al., 2018). 6-phosphofructose-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) has been recognized for its important role in a variety of tumors, and its presence enables glycolysis to be maintained at a high rate (Shi et al., 2017). TGF-β1 induces PFKFB3 expression and stimulates glycolysis and lactate production in pancreatic cancer (Panc1) cells, as well as in GBM (Rodríguez-García et al., 2017; Yalcin et al., 2017). Subsequently, the enzyme pyruvate kinase M2 (PKM2) catalyzes the last step of glycolysis. TGF-β-induced epithelial-mesenchymal transition (EMT) is considered to be associated with glycolysis regulation by mechanically induced PKM2 expression via mTOR/ P70S6K signaling (Cheng and Hao, 2016).

TGF-β contributes to glycolysis, and the glycolysis product lactate, in turn, affects TGF-β. Lactate is accumulated in the microenvironment and acts as a regulator of cancer development (Ippolito et al., 2019). Interestingly, in GBM, lactate promotes the expression of TGF-β2 by upregulating the TGF-β activator THBS-1 (Seliger et al., 2013). In addition, lactate can promote TGF-β from the latent to the active form (Kottmann et

al., 2012). These findings collectively suggest that TGF-β has a positive feedback relationship with glycolysis.

GBM glycolysis affects immune cells through TGF-β

Glycolysis of GBM

There is growing evidence that cancers can alter their metabolic pathways in response to increasing nutritional demands, often through altered gene signals. In GBM, frequent amplification of signals from tyrosine kinase receptor (RTK), especially epidermal growth factor receptor (EGFR), represent one of the core mechanisms of metabolic changes. RTK drives the metastasis of glucose metabolism through downstream PI3K/Akt/mTOR signals, making cancer cells more prone to glycolysis (Agnihotri and Zadeh, 2016; Libby et al., 2018). Elevated MYC levels which occur in most tumors increase glucose flux and glycolytic enzyme expression in GBM (Cascón and Robledo, 2012; Tateishi et al., 2016). The role of MYC in driving glycolysis may be directly or indirectly regulated by mTOR downstream of EGFR (Masui et al., 2013; Liu et al., 2019). The canonical Wnt/β-catenin pathway is also upregulated in GBM, activating glycolysis through its downstream target gene c-Myc (Vallée et al., 2018). The tumor suppressor PTEN is often deleted or mutated in cancer. PTEN deletion causes the activation of Akt, which leads to phosphorylation of phosphofructokinase 1 platelet isoform (PFKP) to increase glycolysis (Lee et al., 2017). As mentioned above, TGF-β signaling and non-Smad signaling pathways including these frequently amplified signals are often cross-communicated (Held-Feindt et al., 2003; Torkamani and Schork, 2009; Zhao et al., 2017). Thus, it is presupposed that they synergistically stimulate GBM glycolysis.

Metabolic reprogramming is not merely the result of genetic alterations, rather, selection for environmental stress is also an important factor. Hypoxia is a hallmark feature of GBM, in which hypoxia-inducible factor-1 (HIF-1) can be activated in response to the decrease in oxygen content. HIF-1 is a heterodimeric protein composed of α (HIF-1α) and β (HIF-1β) subunits, in which HIF-1α plays a key role in the development and progression of GBM (Wang et al., 2017). Activation of HIF-1α induces pyruvate dehydrogenase kinase 1 (PDK1) to reduce GBM mitochondrial respiration and to mitigate cell damage caused by reactive oxygen species (ROS) (Velpula et al., 2013). In addition, HIF-1α stimulates glycolytic energy production through transcriptional activation of the glucose transporter GLUT and enzymes responsible for glycolytic decomposition of glucose, such as HK2 (Wolf et al., 2011; Gillespie et al., 2015). Under hypoxic challenge, the original oncogenic genes such as TGFB1, located downstream of the HIF-1 gene, are upregulated more significantly (Gao et al., 2019). From these we can see that HIF-1α has the dual role of protecting GBM cells from hypoxia-induced damage and increasing GBM's utilization of glucose under hypoxic conditions. However, under normoxia, HIF-1α can be activated by PI3K/Akt, MAPK/ERK, Stat3 and other oncogenic signaling pathways to increase GBM glycolysis (Wang et al., 2017). Therefore, the carcinogenic signal networks promote GBM cells glycolytic metabolic transformation, and TGF-β appears to be an intermediate node in this process.

GBM glycolysis and immune cells

An important mechanism for the maintenance of GBM malignancy is the communication with its surrounding microenvironment, including tumor cells, stromal cells, and immune cells, etc. The immunosuppressive cells infiltrating tumor cells include glioma-related microglia/macrophages (GAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), while the anti-tumor immune cells include T cells (effector T cells, cytotoxic T cells and helper T cells) and NK cells (Grabowski et al., 2021). Immune cells can quickly initiate a host's defense against tumors upon precepting heterogeneous signals. Activated immune cells then utilize multiple cancer-related metabolic pathways, such as glycolysis, to support their rapid transition (Pearce and Pearce, 2013; Huang et al., 2016; Gupta and Dwarakanath, 2017). However, this metabolic adaptation creates a competition between tumor cells and immune cells in the tumor microenvironment (TME) (Gupta and Dwarakanath, 2017). The tumor cells with high metabolism create TME nutrient deficiency, evoking lactate accumulation and hypoxia, all of which lead to inhibitory phenotypes of immune cells. In a nutrient-deficient environment, particularly upon glucose deprivation, T cell proliferation is inhibited due to its high dependence on glycolysis, however Treg cells are not affected (Macintyre et al., 2014). Consistent with T cells are NK cells, which reduce their glycolysis rate after glucose restriction, weakening their cytotoxicity and proliferation (Cong et al., 2018; Terrén et al., 2019). Under acidic conditions, macrophages are polarized from the classically activated M1 type to the alternatively activated M2 type (Ohashi et al., 2017). The production and infiltration of GAMs contribute to the construction of the immunosuppressive environment by either expressing high levels of anti-inflammatory factors (IL-10, IL-6, TGF-β), angiogenic molecules (VEGF-A) and matrix metalloproteinases (MMP9), or inhibiting CD8+ T cell infiltration (Riera-Domingo et al., 2020; Grabowski et al., 2021). Furthermore, hypoxia induces the proliferation of myeloid-derived suppressor cells (MDSCs) (Guo et al., 2018). The hypoxia-induced MDSCs express high levels of programmed death ligand (PD-L1), which, combined with the programmed death receptor (PD-1) on T cells, eventually leads to the inhibition of T cells (Riera-Domingo et al., 2020). In summary, GBM immunosuppression appears to be highly driven by factors in the microenvironment altered

by high intensity glycolytic metabolism.

TGF-β and immune cells

TGF-β is one of the components of the TME and harsh TME promotes tumor cells and surrounding cells to produce additional TGF-β, maintaining it in an active state (Kottmann et al., 2012; Guo et al., 2016). The release of TGF-β not only enhances the tolerance of tumor cells to the TME, but also acts as an effective immune microenvironment regulator (Farhood et al., 2020). TGF-β down-regulates tumor surface antigens such as HLA-DR, NKG2DL, and intercellular adhesion molecule 1(ICAM-1) impairing immune cell infiltration and leading to tumor cells escaping from immune surveillance (Zuber et al., 1988; Eisele et al., 2006; Lohr et al., 2011). TGF-β directly functions on the immune cells in TME, resulting in tumor promotion rather than tumor inhibition. In the early stages of tumorigenesis, the infiltration of NK cells is inhibited by TGF-β signaling (Zaiatz-Bittencourt et al., 2018). However, SMAD4 deficiency affects NK cell control of the tumor due to TGF-β acting through a TβRI-dependent, TβRII-independent atypical pathway (Cortez et al., 2017). TGF-β connects to T lymphocyte exhaustion by regulating multiple biological processes in T cells via Smad3 mediated hinges, especially the activation and metabolism pathways (Kehrl et al., 1986; Campbell et al., 2001; McKarns et al., 2004; Delisle et al., 2013). In addition to the action of T cell proliferation, TGF-β inhibits T-box (T-bet) and Stat4 expression to inhibit T cell initiation and development (Lin et al., 2005). Upon TGF-β-activation, Smads and ATF1 directly bind to the promoter region of the T cell cytotoxic gene, impairing T cell functions (Thomas and Massagué, 2005). Furthermore, TGF-β upregulates the Spred-1 gene, which inhibits the activation of ERK/MAPK pathways and subsequently reduces T cell responsiveness to stimulation (di Bari et al., 2009).

$CD4^+$ $CD25^+$ regulatory T cells (Tregs) are a unique population of suppressor T cells and are increased in most cancers. TGF-β has the ability to induce $CD4^+$ $CD25^-$ immature T cells into Tregs (Chen et al., 2003). In the absence of Samd3, the induction of Tregs by TGF-β is significantly reduced (Martinez et al., 2009). In turn, Tregs inhibit tumor-specific $CD8^+$ T cell cytotoxicity and NK cell function through TGF-β signaling (Chen et al., 2005; Zhou et al., 2010). MDSCs are a group of immature myeloid cells that play an important role in cancer immunosuppression. Tumors promote monocyte differentiation into MDSCs by autocrine action of TGF-β, MDSCs in turn are more efficient at inhibiting T cells and promoting Tregs in the presence of TGF-β (Gonzalez-Junca et al., 2019). Tumor-associated macrophages (TAM) are often associated with poor prognosis of tumors. M1 phenotypic macrophages are induced by the inclusion of TGF-β in TME and differentiate into M2-like phenotypes. This induction mechanism may mean that Smad2/3 and PI3K/AKT signaling pathways are activated to up-regulate transcription factor SNAIL after TGF-β stimulation (Zhang et al., 2016). It may also be related to the inhibition of NF-κB mediated anti-inflammatory response (Porta et al., 2009). TAM itself expresses high levels of anti-inflammatory factors (IL-10, IL-6, TGF-β) that inhibit the function of anti-tumor $CD8^+$ T cells and NK cells (Peng et al., 2017; Cassetta and Kitamura, 2018).

Generally speaking, TGF-β plays an important role in the occurrence and development of gliomas. Tumor cells adapt to changes in the surrounding environment by altering their metabolism, during which they secrete high levels of TGF-β. TGF-β changes the behavior of immune cells and helps tumors to achieve immune escape. Therefore, the TGF-β pathway has also been widely studied as a tumor treatment targets.

TGF-β targeted therapy in GBM

Targeting the TGF-β signaling pathway has become an attractive treatment strategy in cancer. Inhibitors of this pathway have shown good activity in some preclinical or clinical trials (Ciardiello et al., 2020; Teicher, 2021; Liu et al., 2021b). Table 1 summarizes a series of inhibitors of the TGF-β pathway in GBM. In this section, we briefly summarize the progress and application of the TGF-β strategy in GBM, including some small molecule kinase inhibitors, antisense oligonucleotides and neutralizing antibodies.

Many small kinase inhibitors targeting TGF-β receptor kinase have been developed for tumor therapy. Zhang et al. demonstrated for the first time that the TβRI kinase inhibitor LY2109761 increases the radiosensitivity of GBM cell lines and glioma stem cells

Table 1. Selected inhibitors of TGF-β signaling tested for glioma therapy.

TGF-β targeted drugs	Type	Development status	Reference
LY2109761	TβRI kinase inhibitor	Preclinical	Zhang et al., 2011a
LY364947	TβRI kinase inhibitor	Preclinical	Hardee et al., 2012
SB-431542	TβRI kinase inhibitor	Preclinical	Hjelmeland et al., 2004
Galunisertib (LY2157299)	TβRI kinase inhibitor	Phase II	NCT01582269
Trabedersen (AP12009)	TGF-β2 antisense oligonucleotide	Phase III	NCT00761280
Fresolimumab (GC1008)	TGF-β monoclonal antibody	Phase II	NCT01472731
BCA101	EGFR/TGF-β fusion monoclonal antibody	Phase I	NCT04429542

(Zhang et al., 2011a). The growth of mouse subcutaneous xenografts was delayed by either treatment alone or in combination (Zhang et al., 2011b). Other small molecule kinase inhibitors, including LY364947, SB431542, have also been shown to inhibit GBM (Hjelmeland et al., 2004; Hardee et al., 2012). These small molecule inhibitors remain in the preclinical research stage and have not been evaluated in clinical trials. Galunisertib (monohydrate of the small molecule kinase inhibitor LY2157299), an oral drug, has been advanced to a phase II clinical trial for the treatment of patients with recurrent glioblastoma (NCT01582269, clinicaltrails.gov). No medically relevant cardiotoxicity was detected with monotherapy or in combination with lomustine, thus supporting the evaluation of Galunisertib in future clinical trials (Kovacs et al., 2015). Trabedersen (AP12009) is a phosphorothioate antisense oligodeoxynucleotide with specificity for human TGF-β2 mRNA, which has been applied in tumors. A phase II clinical trial was conducted in adult patients with recurrent or refractory anaplastic astrocytoma or secondary glioblastoma. The purpose was to compare the safety and efficacy of 10 μM and 80 μM doses of trabedelsen with standard chemotherapy. The results showed that the total survival time of 10 μM trabedersen was prolonged and the safety was greater than that of standard chemotherapy patients (Bogdahn et al., 2011). To further investigate the efficacy of 10 μM trabedersen, a phase III clinical trial (NCT00761280, clinicaltrails.gov) was carried out, however the trial was prematurely discontinued due to the inability to recruit the expected number of patients.

A monoclonal antibody against TGF-β, fresolimumab (GC1008), has been studied in a Phase II clinical trial. Intravenous injection of GC1008 labeled with 89zr was combined with PET imaging and the therapeutic effect of GC1008 on patients with recurrent malignant glioma was evaluated (NCT01472731, clinicaltrails.gov). The results showed that GC1008 can penetrate recurrent high-grade gliomas well, but it may

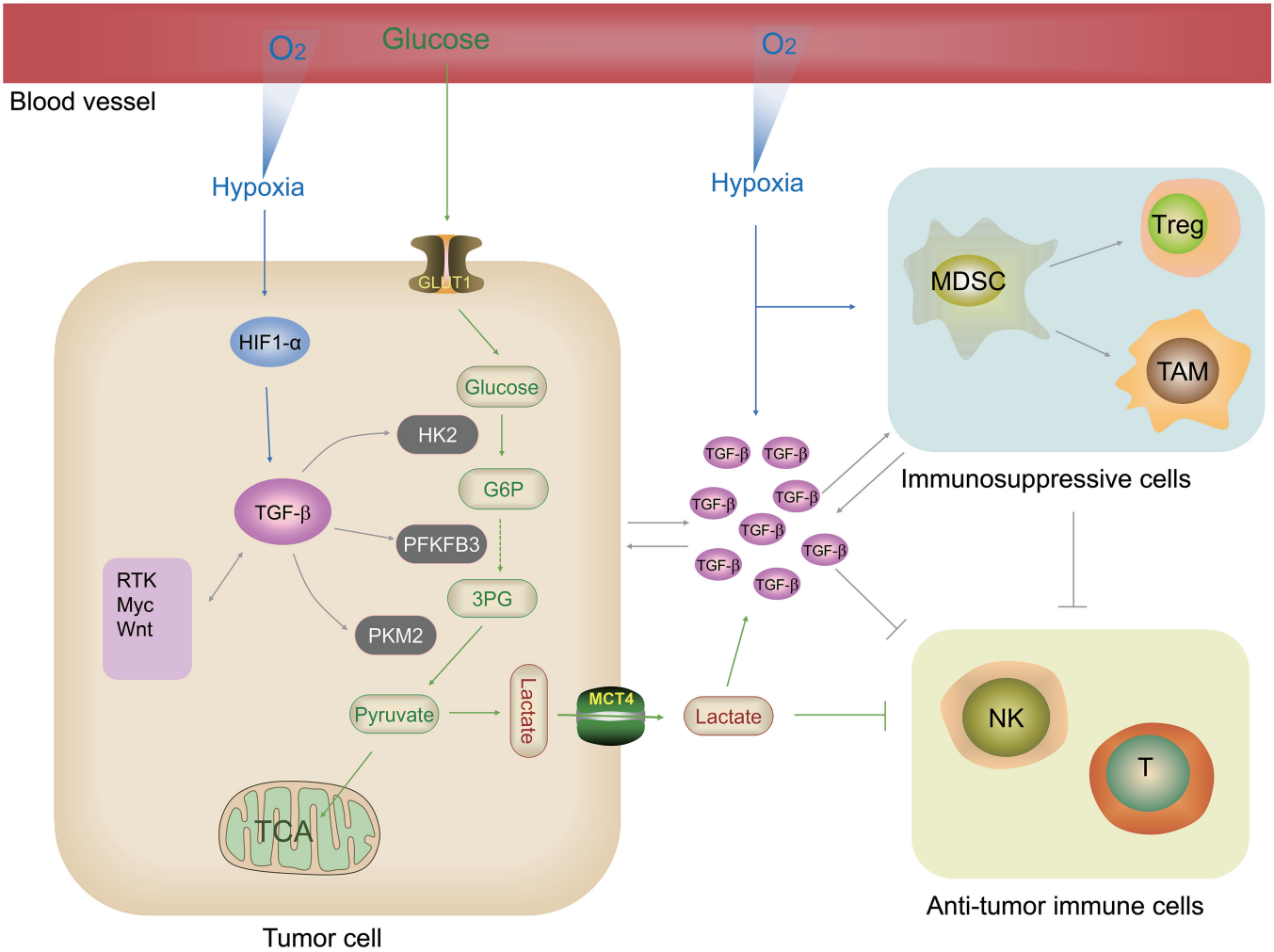

Fig. 4. TGF-β promotes glycolysis of glioblastoma and influences its immune microenvironment.

not bring clinical benefits beyond a single dose. However, the benefits of this treatment at higher doses have been demonstrated previously (den Hollander et al., 2015). BCA101, a bifunctional antibody targeting EGFR and TGF-β, was studied in a Phase I clinical trial in subjects with advanced EGFR-driven solid tumors, including glioblastoma (NCT04429542, clinicaltrails.gov). This clinical trial was first posted in June 2020, no corresponding trial results have been made available and subjects are still being recruited.

Conclusion and perspective

In most cancers, including GBM, TGF-β is highly expressed as an oncogenic factor that promotes proliferation, migration, and immunosuppression. Efforts have been made to reverse this effect by developing inhibitors of the TGF-β pathway. TGF-β has both anti- and pro-cancer effects in the tumor microenvironment, known as the "TGF-β paradox", which is determined by the different stages of cancer development and the background of genomic changes (Seoane and Gomis, 2017). In the early stages of cancer, TGF-β inhibits cell proliferation, making it a cancer suppressor. With malignant transformation of cancer, genomic instability and microenvironmental changes overwhelm the anti-cancer effect of TGF-β. In this review, we discussed that TGF-β signals cooperate with other frequently amplified signals to promote GBM glycolysis, change the GBM microenvironment, and contribute to the immunosuppressive microenvironment (Fig. 4). However, the high molecular and metabolic heterogeneity of GBM leads to a complex and volatile microenvironment. The expression of TGF-β is significantly different in different regions of GBM, especially in the tumor core area and the tumor periphery area (Badr et al., 2020; Manini et al., 2020). The amount of TGF-β presented to cells and the interaction of TGF-β with other factors in the microenvironment can determine its anti-inflammatory or pro-inflammatory response to some extent (Travis and Sheppard, 2014). Therefore, the microenvironmental state is very important in shaping the response of TGF-β, completely inhibiting TGF-β is undoubtedly an imprecise strategy. Future treatment strategies need to consider these complex factors described herein and more personalized treatment plans developed accordingly.

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