Glioblastoma multiforme (GBM) is the most common primary brain malignancy and is often resistant to conventional treatments due to its extensive cellular heterogeneity. Thus, the overall survival of GBM patients remains extremely poor. Insulin-like growth factor (IGF) signaling entails a complex system that is a key regulator of cell transformation, growth and cell-cycle progression. Hence, its deregulation is frequently involved in the development of several cancers, including brain malignancies. In GBM, differential expression of several IGF system components and alterations of this signaling axis are linked to significantly worse prognosis and reduced responsiveness to temozolomide, the most commonly used pharmacological agent for the treatment of the disease. In the present review we summarize the biological role of the IGF system in the pathogenesis of GBM and comprehensively discuss its clinical significance and contribution to the development of resistance to standard chemotheraphy and experimental treatments.

Keywords: insulin/insulin-like growth factor system, insulin-like growth factor signaling pathway, IGF-binding protein, glioblastoma, drug resistance

INTRODUCTION

Malignant gliomas represent 30% of all intracranial tumors and include a heterogeneous group of neoplasms that arise from multiple cell types with neural stem cell-like properties (1). In the US, the annual incidence of gliomas is 3.2 cases for every 100,000 individuals and about half of them are classified as glioblastoma or glioblastoma multiforme (GBM) (2). Incidence increases with age and is more common in men than in women and in individuals of caucasian ethnicity (3). Disease prognosis is poor, with a 35% survival rate at 1 year and <5% at 5 years (4). GBM can be classified as primary when it develops within a few months without known precursor states, or secondary, when a low-grade tumor evolves in a GBM. Surgery, radiation therapy and chemotherapy with alkylating agents such as temozolomide (TMZ) represent the mainstay of GBM treatment (5). Additionally, several small molecules have been tested in the last few years (6, 7). However, none of these strategies represent an effective cure for GBM, as this malignancy displays extensive intra-tumoral heterogeneity that favors disease recurrence (8, 9). Genomic profiling carried out by The Cancer
Genome Atlas (TCGA) consortium on 200 GBM samples as well as a complementary study by Pearson and colleagues revealed recurrent genetic alterations involving TP53, retinoblastoma (Rb) and different receptor tyrosine kinases (RTK) pathways (10, 11).

RTKs are a family of cell surface receptors, comprising the epidermal growth factor receptor (EGFR), the fibroblast growth factor receptor (FGFR), the hepatocyte growth factor receptor (HGF/c-MET), the platelet-derived growth factor receptor (PDGFR), the vascular endothelial growth factor receptor (VEGFR) and the insulin-like growth factor 1 receptor (IGF-1R). The latter is part of the insulin and insulin-like growth factor (IGF) family that includes three ligands (insulin, IGF-I and IGF-II), different cell surface receptors (the insulin receptor, the IGF-1R, IGF-IIR and hybrid heterodimer receptors between the insulin receptor and IGF-1R) and six IGF-binding proteins (IGFBP 1 to 6). This complex system promotes the release of IGFs modulating their interaction with receptors and different IGFBP proteases (12–15).

In the extracellular compartment, insulin and IGFs activate intracellular signaling pathways by binding—with different affinity—their cognate and/or non-cognate receptors. The interaction between ligands and receptors results in the recruitment of downstream insulin receptor substrates (IRSs) and SRC homologous and collagen-like (SHC) proteins. These cytoplasmatic proteins modulate the activation of the PI3K/AKT/mTOR axis and the RAS/RAF/MEK/ERK pathway involved in the transcription of genes regulating cell proliferation, cell-cycle progression, cell motility and apoptosis (16).

Aberrant activation of the Insulin/IGF signaling plays a crucial role in dysregulation of multiple cellular pathways in different tumors and its atypical activation is usually associated with a poor prognosis (17–21).

In the present review we summarize current research on the role of the IGF system in the pathogenesis of GBM and discuss the clinical significance and therapeutic implications of this pathway in the development of resistance to both currently approved and experimental treatments.

**EXPRESSION OF IGF SYSTEM COMPONENTS IN GBM**

Studies on the expression of Insulin/IGF system components in human GMB samples are often conflicting and depend on the detection technique employed.

Use of in situ hybridization (ISH) or immunohistochemistry (IHC) techniques for the detection of IGF-I and II revealed high levels of these two ligands in GBM samples compared to normal glial tissue (22–25). On the contrary, analyses conducted by real-time PCR on tissue microarrays showed no change in IGF-I or IGF-II transcripts between normal glial samples and high-grade gliomas, including GBMs (23, 25).

Both IGF-IR and IGF-IIIR are overexpressed in GBMs compared with normal brain and this overexpression is often associated with inferior survival and a less favorable response to therapy (23, 26).

IGFBPs overexpression is also well documented in glioblastoma. Different studies demonstrate that IGFBP-2 is overexpressed in glioma cells showing a distinct progression-related expression change from low- to high-grade gliomas (27–29). Indeed, high IGFBP-2 levels promote both proliferation and invasion of glioma cells and have been linked to an adverse prognosis in high-grade gliomas (30–32).

IGFBP-3, IGFBP-4 and IGFBP-5 transcripts are significantly higher in GBM compared to low-grade gliomas or normal samples, supporting their role in the pathogenesis of gliomas. Furthermore, IGFBP-4 up-regulation favors epithelial-mesenchymal transition (EMT), proliferation, migration and invasion (29, 33, 34). Unlike other IGFBPs, the expression of IGFBP-6 inversely correlates with glioma grade and higher plasma IGFBP-6 levels have been associated with a better prognosis (15, 35).

Therefore, dysregulation of several IGF system components is usually associated with a more aggressive disease displaying poor response to therapy and a shorter survival (15).

**THE IGF SIGNALING PATHWAY IN GBM**

IGF-I interaction with IGF-IR appears to trigger low-grade glioma progression to GBM (Figure 1A). Specifically, IGF-IR stimulation by IGF-I promotes glioma cell proliferation and migration by negatively or positively modulating PI3K/AKT signaling through a mechanism conditioned by a specific cellular context (36, 37). In a subpopulation of glioma stem cell-like cells identified as recurrence-initiating stem-like cancer (RISC) cells, IGF-IR maintains cell survival through an autocrine activation that downregulates both AKT and ERK signaling leading to slow-growth but high-self-renewal (38, 39). On the contrary, this mechanism is not observed in non-glioma stem cells. Furthermore, Hagerstrand and colleagues demonstrated that glioma cells showed ligand-independent AKT phosphorylation and that combined inhibition of IGF-IR and PI3K or mTOR reduces cell viability (37). These data are also supported by recent evidence suggesting that, IGF-I or IGF-IR overexpression causes AKT phosphorylation while its inhibition reduces IGF-mediated anti-apoptotic effects (40).

Involvement of the AKT pathway in GBM pathogenesis is also connected to activation of the insulin receptor (InsR) that promotes proliferation and survival of glioma cells both in an insulin-dependent and -independent manner (Figure 1A). Insulin signaling is robustly activated in human glioma cells independently of insulin stimulation, since the catalytic subunit of the InsR strongly phosphorylates and recruits IRS-1 leading to the activation of AKT and ERK2 (41) (Figure 1A). Furthermore, experiments conducted in insulin-stimulated IRS-1-transfected glioma cell showed high phosphorylation levels of AKT, ERK1/2 and overexpression of Grb2 resulting in increased cell viability (42).

The compensatory mechanism within the InsR/IGF signaling network was widely described in different tumor types (43, 44).
FIGURE 1 | Expression and function of the insulin/insulin-like growth factor system in glioblastoma cells. (A) The pathogenesis and progression of glioblastoma multiforme (GBM) is mainly linked to the activation of insulin-like growth factor 1 receptor (IGF-IR) and insulin receptor (InsR). The stimulation of IGF-IR and the activation of InsR promote the recruitment of IRS-1 and the activation of the mitogenic and pro-survival mediators Akt and ERK1/2 that contribute to increase (upward red arrow) GBM cell growth, cell proliferation and cell migration. (B) A pivotal role in regulating IGF-system action in GBM pathogenesis is provided by IGFBP-2. (1) The improper activity of protease that cleaves IGFBP-2 causes an increase of circulating-free IGFs (upward red arrow) and the subsequent activation of downstream pathway causing an increase on cell growth, cell proliferation and cell migration. The intracytoplasmic and intranuclear activity of IGFBP-2 determines the activation of EGFR-STAT-3 (2) or HIF1a (3) signaling and the improvement of the transcription of their target genes. IGFBP-2 activates also the integrin signaling (4) which lead to the activation of the IGFBP-2/Integrin/ILK/NF-kB pathway that is responsible of the transcription of genes linked to adhesion and migration. Finally, stimuli induced by IGF-I, NF-kB, HIF1a, steroid and growth hormones (5) increase IGFBP-2 expression (upward red arrow) causing the activation of pathway linked to GBM progression.
Gong and colleagues found predominant InsR mitogenic isoform-A in GBM surgical specimens and xenograft tumor lines. Interestingly, they observed that InsR-A depletion compromised Akt activation repressing orthotopic tumor growth, but this event has been restored by stimulated IGF-IR expression. These data confirm the cooperation between InsR and IGF-IR also in glioma cells, suggesting a compensatory crosstalk addressed to balance potential defective activity in one of two signaling (45).

Crosstalk between InsR/IGF systems and other RTK pathways has been demonstrated in many human cancers, including GBM (46–50). It was reported that Hedgehog-IGF-I crosstalk preserves the self-renewal properties in GBM. This interaction causes VEGF expression and increases survival and proliferation in Glioma Stem Cell (GSC) involving Gli1 but not Gli2 proteins (51, 52). A crosstalk between InsR/IGF-IR and EGFR linked to an Akt-mediated compensatory intracellular mechanism was also described (53).

IGFBPs have multiple and complex functions playing a pivotal and significant role in regulating IGF-system action in GBM. Different authors reported IGFBP-2 as central mediators in GBM pathogenesis and two controversial mechanisms have been hypothesized (Figure 1B). The first one includes an improper protease activity of IGFBP-2 that determines an increase of circulating-free IGFs and the activation of EGFR-STAT3 signaling in an IGF-independent manner, involving the intracytoplasmic and intranuclear IGFBP2 functions (54). The second mechanism is more complex. In GBM cells IGFBP-2 expression is inversely correlated with PTEN levels, thus promoting Akt-mediated signaling. In addition, stimuli induced by IGF-I, NF-kB, hypoxia-inducible factor 1a (HIF1a), steroid and growth hormones increase IGFBP-2 expression eventually causing GBM progression. Furthermore, increased IGFBP-2 levels have been directly associated to enhanced cell proliferation and correlates with HIF1a-mediated stimulation of VEGF pathway (55). Moreover, IGFBP-2 and integrin alpha5 interaction seem necessary to promote glioma cell migration in a JNK-dependent manner (56). Finally, IGFBP-2 was also identified as critical component of a complex network (IGFBP-2/Integrin/Integrin-Linked Kinase (ILK)/NF-kB) able to drive glioma progression. Kristen and colleagues proposed that IGFBP-2 is request for the formation of integrin complex which lead to ILK and NF-kB activation responsible of diffuse glioma progression (27).

As well as IGFBP-2, also IGFBP-3 improves glioma cell migration, invasion and proliferation in an IGF-independent manner. The tumor promoting properties of IGFBP-3 are linked to its ability to increase STAT-1 expression that, in turn, result associated with reduced patients overall survival (OS) (57). Furthermore, Chia-Hua Chen demonstrated that IGFBP-3 silencing suppresses glioma cell proliferation by G2/M cell cycle arrest (58). On the contrary, the anti-tumorigenic and anti-angiogenic functions of IGFBP-4 in glioma cells depend to its ability to induce dibutylryl cyclic AMP (dB-cAMP) that antagonize the VEGFs action in an IGF-independent manner (59).

IGFBP-5 is highly expressed in GBM, indeed its depletion results in an inhibition of cell invasion and concomitant increase in cell proliferation. The double role of IGFBP-5 is mechanistically associated with Akt and EMT signaling (60).

Lastly, IGFBP-6 is a member of a paracrine signaling circuit involving the IGF-II-IGF-IR axis present in TMZ-resistant glioma cells. IGFBP-6, released by TMZ-sensitive cells, reduces the expansion of the counterpart resistant cell population by sequestering IGF-II that results in IGF-IR-Akt signaling inactivation (15).

MicroRNAs As Regulators of the IGF System in Gbm

MicroRNAs (miRNAs) are a class of small non-coding RNAs that are usually classified into tumor-suppressive miRNAs (TS-miRNAs) and onco-miRNAs depending on their ability to suppress or favor tumorigenesis. MiRNA dysregulation is associated with initiation and progression of several forms of cancer, including GBM (61, 62).

It has been reported that miRNA miR-128, miR-422a and miR-603 inhibit IGF-I expression in glioma cells as overexpression of these miRNAs suppresses cell proliferation, migration and invasion (63) and enhances apoptotic death through the inhibition of the mTOR signaling pathway (64). Furthermore, a plethora of TS-miRNAs (including miR-7, miR-15b, miR-181b, miR-186, miR-320a, miRNA-323-5p, miR-383, miR-422a, miR-503, miR-505, and miR603) target IGF-IR messenger RNA in the non-neoplastic brain, thereby acting as tumor-suppressors. In GBM the expression of these miRNAs is reduced resulting in activation of the IGF-IR/AKT axis. In vitro studies demonstrated that TS-miRNAs overexpression also induces cell-cycle arrest and apoptosis (63, 65–74). Several miRNAs modulate IGF/IGF-IR signaling by targeting its downstream effectors. Indeed, miR-204-3p and miR-491-3p inhibit IGFBP-2 expression and are downregulated in glioma cells (75, 76). MiR-302b indirectly inhibits IGFBP-2 activity by downregulating the expression of its direct target Nuclear factor IA (NFIA), which transcriptionally regulates IGFBP-2 expression (77). MiR-7 potently inhibits EGFR, IRS-1 and IRS-2 expression (78), while the brain-specific miR-153 downregulates IRS-2 (79). Moreover, the tumor suppressor miR-128-3p indirectly regulates IRS–1 expression by modulating the expression of Neuronal Pentraxin 1 situated upstream of the IRS–1/PI3K/akt pathway (80). The reported downregulation of these TS-miRNAs in malignant glioma results in IRS overexpression and activation of the PI3K/akt pathway (78, 79).

Finally, among the onco-miRNAs, only miR-21 and miR-513a-5p are overexpressed in human GBM cell lines and tumor tissue (81, 82). Yang and colleagues reported that high levels of miR-21 lead to downregulation of IGFBP-3, restraining its antiproliferative activity. Interestingly, miR-21 expression levels are inversely correlated with GBM survival (82).
THE IGF SYSTEM AND TREATMENT RESISTANCE IN GBM

Several pre-clinical evidences suggest that the IGF system plays a pivotal role in the development of resistance to chemotherapy, radiation and targeted therapies, eventually resulting in GBM recurrence and/or progression (83).

Differences in both protein expression and miRNA production related to the IGF pathway have been found in TMZ-sensitive and -resistant GBM cells. These differences could be exploited to restore chemosensitivity in resistant cells. Indeed, compared with TMZ-sensitive malignant glioma cells, the resistant ones express higher miR-497 levels. This difference may play a role in the induction of TMZ resistance through the activation of the IGF-IR/IRS-1 pathway-related proteins, that are IGF-IR, IRS1, mTOR and Bcl-2 (84). Furthermore, TMZ-resistant cells display lower IGFBP-6 expression than TMZ-sensitive cells, with the latter down-regulating both IGF-II and IGF-IR as IGFBP-6 acts as a suicide substrate binding IGF-II with high affinity and preventing its interaction with IGF-IR. Although the site of IGFBP-6 endogenous production remains unclear, this model suggests a paracrine secretion from TMZ-sensitive cells. Hence, by depleting the sensitive population, TMZ selects the resistant one, ultimately promoting growth of the tumor mass (15, 84). The IGF-system also seems to determine resistance to alkylating agents through a miRNA-mediated activation of WNT/β-catenin signaling. In detail, IGF-1 up-regulates miR-513a-5p, which in turn represses NEural precursor cell-expressed Developmentally Downregulated 4-Like (NEDD4L), ultimately leading to WNT/β-catenin activation (81). NEDD4L is a tumor suppressor gene encoding for a ubiquitine ligase, which regulates ion channel expression and intracellular signaling (85). Indeed, low NEDD4L levels correlate with TMZ resistance and poor prognosis in gliomas (86).

The induction of a stem-cell phenotype is one of the main causes of resistance to radiation therapy in GBM. IGF signaling is involved in this process through mechanisms only partially understood (39, 87). MiR-603 targets IGF-I and IGF-IR, facilitating the exit from the stem-cell condition and conferring sensitivity toward radiation. However, ionizing radiation reduces miR-603 expression by enhancing its extracellular vesicle-mediated export. Hence, lower levels of MiR-603 induced by radiotherapy may promote a stem-cell state. miR-603 also suppresses O6-Methylguanine-DNA-Methyltransferase (MGMT). Consequently, decreased miR-603 activity determines MGMT up-regulation, which ultimately leads to cross-resistance toward alkylating agents in glioma cells (74). Recently, Simpson et al. further supported the involvement of the IGF-IR in the radiation resistance of high grade gliomas describing radio-sensitization after IGF-IR inhibition in pediatric patients (88).

Even though the EGFR represents a potential target for GBM treatment, its inhibition led to dismal results due to intrinsic tumor resistance to this approach (89, 90). Indeed, activation of a compensatory IGF pathway exerts a pivotal role in the lack of sensitivity toward EGFR inhibition. Simultaneous targeting of the EGFR and IGF-IR was effective in GBM cell lines and patient derived xenografts (PDX) (53, 91). On a different note, miR-7 overexpression sensitizes GBM cells to the antitumor effect of erlotinib via blockage of the IRS/AKT pathway (92). Thus, quantification of IGF-IR tissue levels may be employed as a predictive biomarker to improve the selection of GBM patients likely to benefit from EGFR inhibitors as reported in the NCT00897663 trial.

The PDGFR is another RTK involved in gliomas development. As for EGFR, PDGFR inhibition has marginal activity in GBM (89, 93, 94). Again, the InsR and IGF-IR seem key effectors of resistance to PDGFR inhibitors and combining an anti-PDGFR and an anti IGF-IR decrease the viability of primary mouse tumor sphere PDGFR-resistant clones obtained from glioma samples in vitro (95).

Inhibition of key components within the GBM tumor microenvironment may also represent an attractive strategy. To this end, colony-stimulating factor-1 receptor (CSF-1R) inhibitors, which block the tumor-associated macrophages and microglia, are currently under development (96, 97). Quail and colleagues investigated the mechanism of resistance to CSF-1R inhibition in murine models of GBM, discovering that it is mediated by activation of the PI3K pathway via the IGF-1/IGF-IR system. Hence, coupling CSF-1R and PI3K or IGF-IR inhibition elicits a sustained response in mice (98).

Recently, a molecule targeting the sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) has been tested on patient-derived GBM neurospheres, identifying responder and non-responder cell lines. The latter seem to bypass SERCA activity via a mechanism mediated by IRE1 (derived from ERN1 gene), IGFBP-3 and IGFBP-5. Indeed, CRISPR-mediated deletion of the ERN1, IGFBP3, IGFBP5 genes in a human GBM cell line increased responsiveness to drugs (99).

ANTI-IGF THERAPEUTIC STRATEGIES IN GBM

Despite the many advances in the field of molecular targeted therapy, the use of small molecules agents or monoclonal antibodies for the treatment of GBM is extremely limited and some promising pre-clinical results have yet to translate into meaningful therapeutic benefits (100–103).

Many approaches have been tested to inhibit IGF signaling in GBM including anti-sense oligonucleotides, tyrosine kinase inhibitors (TKIs) or monoclonal antibodies targeting the IGF-IR (Figure 2). In a pilot study, inhibition of IGF-IR expression by antisense strategy failed to produce any clinical benefit for patients with recurrent GBM (104). An improvement was obtained by employing the combination of IGF-1 antisense/triple helix vector strategy to create immunogenic cells that were injected in GBM patients where they induced an antitumor immune response and stopped tumor progression. The survival of two treated GBM patients reached 2 years (105, 106).
Promising results were also obtained with a treatment based on autologous glioma cells treated \textit{ex vivo} with an antisense oligodeoxynucleotide targeting the IGF-IR (IGF-1R/AS ODN) and re-implanted in patients. Seventeen out of 33 individuals enrolled in a phase Ib trial (NCT02507583) remained progression-free and 12 of them are still alive, demonstrating that an autologous cell vaccine may significantly prolong progression free survival (PFS) and OS compared to standard of care (107).

Several IGF-IR-directed TKIs have been tested in GBM patients with promising pre-clinical results. PQ401, GSK1838705A and PPP (picropodophyllin/AXL1717) all reduced cell viability and proliferation \textit{in vitro}, while the administration in mice led to inhibition of glioma tumor growth \textit{in vivo} (26, 108, 109). Notably PPP causes dramatic tumor regression not only in subcutaneous but also in intracerebral xenograft models, indicating that it crosses the blood-brain barrier. Hence a phase I/II clinical trial was initiated (NCT101721577) but results have not yet been posted. NVP-AEW541 is another IGF-IR-specific TKI that in combination with dasatinib significantly increases BAX activation inducing more apoptosis than either agent alone in glioma cells (110).

Multiple IGF-IR blocking antibodies are currently in clinical development (111) but - to date - only cixutumumab (IMC-A12) has been clinically tested on patients diagnosed with GBM. Cixutumumab binds IGF-IR or IGF-IR/IR hybrid receptors blocking the interaction with their ligands and inducing receptor internalization and degradation. Zamykal and colleagues demonstrated that cixutumumab inhibits GBM progression in \textit{in vivo} xenograft models (112). A phase I study of IMC-A12 in combination with temsirolimus was also conducted in a group of pediatric patients including four with refractory GBM demonstrating that this combination was well tolerated (113). However these results were deemed insufficient to pursue further development of this compound.

CONCLUSIONS

GBM is an aggressive disease, with a dismal prognosis and scarce therapeutic resources. Hence, understanding the biological features underlying disease pathogenesis, maintenance and progression represents urgent and unmet medical need. To date, a plethora of pre-clinical evidence demonstrates a crucial role for the IGF system in GBM. Altered IGF signaling as well as cross-talk with multiple pathways are complex and far from being fully understood. However, the IGF system could potentially be exploited for both diagnostic and therapeutic purposes. For example, the expression level of many components of the IGF signaling axis or their serum concentration in GBM patients may become prognostic biomarkers, eventually guiding therapeutic choices. Unfortunately, thus far the
clinical efficacy of treatment strategies directed against the IGF system remains limited and inconsistent. Inter- and intra-tumor heterogeneity, cross talk between IGF and RTK signaling and limited drug distribution to the central nervous system, are some of the potential reasons for the lack of substantial anti-cancer activity. Hence, further development of innovative therapeutic approaches is warranted in order to expand the therapeutic armamentarium for GBM patients.

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AUTHOR CONTRIBUTIONS

Conceptualization, ET and MM. Formal analysis, ET and SG. Data curation, ET and CT. Writing—original draft preparation, ET, MM, MP, SS, FM, GP, CR, AP, and LM. Writing—review and editing, ET, MM, MP, SS, FM, AP, GP, CR, SV, and LM. Supervision, PV. All authors contributed to the article and approved the submitted version.
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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