Chapter 3

Receptor Tyrosine Kinase Interaction with the Tumor Microenvironment in Malignant Progression of Human Glioblastoma

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Abstract

Glioblastoma (GBM) is the most malignant brain tumor, characterized with a rapid progression and poor prognosis despite modern therapies. Receptor tyrosine kinase (RTK) is a membrane tyrosine kinase that could be activated by binding ligands with the extracellular domain, and communicating signals according to the tyrosine kinase activity of the intracellular domain. Recent studies revealed that RTKs such as EGFR, PDGFR and MET play key roles in cancer progression through regulation of abundant cellular processes. As transmembrane proteins, RTKs work as a mediator between the extracellular environment and intracellular compartments, translating the tumor microenvironment (TME) signals into the tumor cells. TME is also a critical regulator for the malignant process, lately receiving considerable attention. It is composed of extracellular matrix (ECM), the stromal cells (i.e., endothelial cells, microglia and fibroblasts), secreted factors, and hypoxia environment, etc. Among these, the strong invasion and sustained angiogenesis of GBM are closely related to ECM-receptor interaction and -associated signaling events. In this chapter, we consider the interaction and mechanisms of RTKs and TME in GBM progression, especially the role of ECM-receptor mediated signaling in tumor invasion, hypoxia and angiogenesis, glioma stem cells and tumor metabolism. We then summarize and discuss recent improvements on the approaches of targeting RTK and TME as the therapy in the primary GBM.

Keywords: glioblastoma, receptor tyrosine kinase, tumor microenvironment, extracellular matrix, focal adhesion complex, signal transduction, invasion, angiogenesis

1. Introduction

Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults. Recently, based on mutations in the gene encoding isocitrate dehydrogenase enzyme 1/2(IDH1/2), GBMs were separated into three main groups (2016 WHO classification of CNS tumors): (1) IDH-wild-type GBMs (about 90% of cases); (2) IDH-mutant GBMs (about 10% of cases); and (3) not otherwise specified (NOS) GBMs. Among these, IDH-mutant phenotype is strongly associated with secondary GBM, younger age, and better outcome, while IDH-wild-type with primary GBM. Typical molecular alterations in primary GBM include mutations in genes regulating receptor tyrosine kinase (RTK)/rat sarcoma (RAS)/phosphoinositide 3-kinase (PI3K), p53, and retinoblastoma protein (RB) signaling.

There have been identified approximately 58 mammalian RTKs, which contain an intracellular catalytic protein tyrosine kinase domain and regulatory sequences, transmembrane domain, and an extracellular ligand-binding domain [1]. In response to environmental cues, RTKs are crucial regulators of the growth factor signaling that controls cellular processes including proliferation, metabolism, survival, etc. RTK activation triggers complex signaling network through Ras/Raf/MEK/ERK, PI3K/Akt and other intracellular pathways in both physiological and pathological conditions; RTK dysregulation through mutation and amplification often occurs in a wide range of cancers including GBMs. RTKs such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), c-Met, Tie, Axl, discoidin domain receptor 1 (DDR1), erythropoietin-producing human hepatocellular carcinoma (Eph) and others play a major role in human GBM pathobiology [2]. Therefore, RTK-targeted agents including tyrosine kinase inhibitors and antibodies are currently used in preclinical and clinical settings in cancers including GBM.

The tumor microenvironment (TME) in malignant glioma is a dynamic entity that consists, besides glioma cells [including glioma stem cells (GSCs)], of an intricate network that encompasses various cell types (e.g., endothelial cells, astrocytes, microglia, and pericytes), stromal components, soluble factors, as well as the extracellular matrix (ECM) [3, 4]. Together, these TME elements play an important role in facilitating the integration of tumor cells with their surrounding environment maintaining features of tumor malignancy [3]. Initially, tumor cells actively exploit their stromal environment through the recruitment of nonmalignant cells and elements that may provide physiological resources to facilitate rapid tumor growth. In time, these recruited cells become a major source of secreted factors to mobilize further inflammatory cells into the microenvironment until the entity becomes steady and strong to progression [5]. In the meantime, rapid proliferation of the malignant cells per se has a metabolic effect on the TME, which is rapidly deprived of glucose and oxygen, becoming acidic and hypoxic [6]. Overall, both tumor cells and the TME are adaptive and undergo evolution from time to time during tumor progression. Human brain tumor bears unique TME in that the tumor rarely metastasizes to other parts of the body [7]. Currently, almost in each type of cancer, TME has drawn much attention regarding the mechanisms of cancer biology and novel therapeutic strategies.

In this chapter, we consider the interaction and role of RTKs and TME during GBM progression; especially their close interactions in GBM biology and targeted therapies. We then discuss recent improvements on approaches of targeting RTKs and TME mainly in primary GBM with IDH-wild-type.

2. RTK activation is a hallmark of malignant glioma

2.1. Genetic alterations of RTK in primary GBM

Aberrant RTK activation frequently occurs during glioma initiation and progression and that the associated activation cascades may cooperate through multiple signaling cross-talks in the malignant transformation of cells, tumor growth and progression, treatment resistance, and disease relapse. In 2008, the Cancer Genome Atlas project (TCGA) reported significant alterations in three core signaling pathways, including RTK/RAS/PI3K (88%), p53 (87%), and retinoblastoma protein (78%), in the collected samples from patients with primary GBM, which may represent the majority of human GBM [8]. 60% of the primary GBM harbors RTK amplifications and/or mutations, among them, EGFR amplifications and/or mutations were observed more than 50% of the disease. About half of GBM with EGFR amplification had an in-frame deletion of exons 2–7 from the extracellular ligand-binding domain of EGFR resulting in a mutant protein with ligand-independent receptor activity (designated delta-EGFR or EGFRvIII) [9]. Therefore, EGFRvIII is commonly expressed in a subset of EGFR-amplified cells. Only a small portion (7%) of the tumor showed EGFR genetic alterations in combination with other RTK lesions. Amplification of platelet-derived growth factor receptor alpha polypeptide (PDGFRA) occurs in 13% of GBMs; ErbB2 (HER2/Neu) belongs to the EGFR receptor family that includes the other three members: EGFR, ErbB3, and ErbB4. Activation of ErbB2 depends on the patterns of dimerization within other family members [10]. ErbB2 mutation was observed in 8% GBM tested in a TCGA study [11, 12]. MET amplifications and fibroblast growth factor receptor (FGFR) mutations, including fusion genes, occur in about 2% of the GBMs [1]. Additionally, overexpression of ligand and/or receptor and co-expression of both (autocrine loop formation) are frequent events in cancers, including GBM, and many have been associated with increased malignancy and worse patient outcome.

2.2. Cooperation of RTKs and their downstream signaling pathways

RTK alterations usually coexist with mutations that activate other core regulatory pathways, including intracellular Ras/MAPK and PI3K/Akt pathways, as well as tumor suppressor pathways in certain types of GBM. Furthermore, the frequent co-occurrence of mutations in *PI3K* and deletion of *PTEN*, in addition to the co-occurrence of mutations and/or deletion of cyclin-dependent kinase inhibitor 2A (CDKN2A; encoding both INK4A and ARF) were observed within all of the detectable RTK alterations in primary GBM. This is consistent with the required cooperation of multiple core pathways for tumor formation in genetically engineered mouse models of GBM. Besides, phosphorylated tyrosine kinases of RTK provided PLC-γ1 docking sites for PLCG1 SH2 domains, leading to phosphorylation of tyrosine kinases on PLC-γ1 and signaling activation pathways [13, 14]. JAK/STAT3 signaling was reported associated with EGFR and EGFRvIII signaling [15].

Given that individual tumor cells express multiple RTKs, it is reasonable to speculate that these RTKs are actively interacting with each other. For example, the phosphorylation of c-Met receptor is strongly correlated with functional levels of EGFRvIII, suggesting the presence of cross-talk between these two RTK signaling, although the intermediary molecules were not elucidated [16]. The Axl RTK follows a similar phosphorylation response as a function of EGFRvIII levels [16]. EGFRvIII expressed in glioma cells stimulates upregulation of TGFα and

HB-EGF, which stimulate in turn wild-type EGFR forming an autocrine loop [17]. It was previously reported that EGFR and EphA2 are both expressed in GBM cells and co-localize to the cell surface. EphA2 phosphorylation is dependent on EGFR activity, and EphA2 downregulation inhibits EGFR phosphorylation, downstream signaling, and EGF-induced cell viability [17]. HGF indirectly activates alternative RTKs such as EGFR by upregulating expression of EGFR ligands such as TGF-α and HB-EGF [2]. Previous studies report that EphA4, whose expression is correlated with increasing glioma grade, forms a heteroreceptor complex with fibroblast growth factor receptor 1(FGFR1) in glioma cells and that the EphA4-FGFR1 complex potentiated FGFR-mediated downstream signaling such as Akt/MAPK, Rac1, and Cdc42 pathways, resulting in the promotion of invasion [18]. A few other reports suggest that Tie2 activation regulates angiogenesis in a highly context and tissue-dependent manner and closely collaborates with VEGF and other angiogenesis regulators [19, 20].

2.3. Heterogeneity of RTK expression within the TME

Human GBM is characterized with high degrees of intertumoral and intratumoral heterogeneity. For example, individual GBM tumors display striking histological variations. As a hallmark of GBM development, oncogenic RTK activation is highly responsible for malignant behaviors of multiple cells in the TME other than GBM cells, that is, endothelial cells, epithelial cells, astrocytes, infiltrated immune cells, glioma stem cells (GSC), etc. [2]. The malignant grade in human astrocytoma was associated with an upregulation of the PDGFRβ on vessel endothelial cells indicating the role of paracrine activation in tumor angiogenesis [21, 22]. Besides EGF, five other respective ligands activate EGFR including transforming growth factor alpha (TGF-α), amphiregulin, betacellulin, heparin-binding EGF-like growth factor (HB-EGF), and epiregulin, respectively. These ligands are secreted by glioma cells and received by tumor microenvironmental cells such as microglia and reactive astrocytes [2]. Axl/Gas6 signaling has multiple functions to regulate survival, proliferation, and migration in a variety of cells in vitro including tumor-derived cell lines of epithelial, mesenchymal, and hematopoietic origin [23]. Moreover, the Eph/ephrin system plays a role in many biological processes such as cell adhesion and migration during development, especially in the central nervous system [24]. In glioma, different Eph receptors are overexpressed not only in tumor cells but also in the surrounding tumor-infiltrating cells like tumor-associated macrophages (TAMs) [25], endothelial cells, stromal cells [26], as well as GSCs [27].

Activation of RTK pathways can lead to cellular transformation and result in genetic alteration in GSCs. Fully differentiated neural cells were able to generate malignant glioma upon PDGFA overexpression and showed high expression of stem and progenitor cell markers [28]. Growth factors such as PDGF, bFGF, and EGF were usually added to the serum-free media to maintain properties of cancer stem cells derived from patient tumor biopsies [29]. HGF/c-Met pathway was involved in brain tumorigenesis and malignant progression, and thus, HGF/c-Met signaling may maintain GSC properties [30]. Moreover, RTKs show various regional expression pattern within tumor in situ during tumor progression, for example, histopathological analysis on in vivo human glioma biopsies showed that Ang-2, MMP-2, MT1-MMP, and laminin5γ2 are co-overexpressed in the invasive areas but not in the central regions of the glioma tissues [31]. GBM is characterized with the unique pattern showing that necrotic areas are typically surrounded by "pseudopalisading" glioma cells, which are highly

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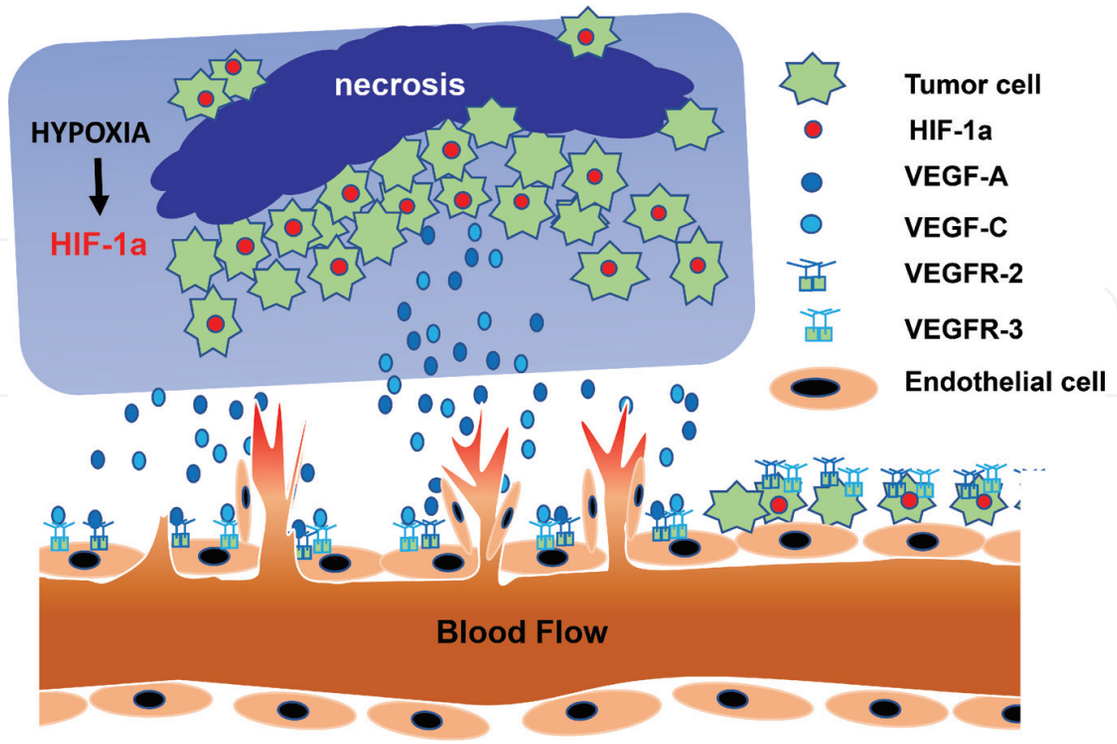

Figure 1. The hypoxic tumor cells stimulate neovascularization in GBM. Under hypoxic conditions, tumor cells secrete enhanced levels of VEGF family members (VEGF-A, VEGF-C). Endothelial cell-specific RTKs (VEGFR-2,VEGFR-3) via ligand (VEGF-A, VEGF-C) binding to stimulate proliferation and migration of endothelial cells. The peri-vascular regions contain glioma stem cells (GSCs).

hypoxic (**Figure 1**). Axl is predominantly expressed in the pseudopalisading cells, along with other markers such as VEGFR, etc. Furthermore, an accumulation of Axl positive tumor cells appeared adjacent to microvascular neoformations, which is a characteristic feature of invading glioma tumor cells spreading along perivascular regions [2].

3. Active interactions between RTK and TME

3.1. RTK, hypoxia and angiogenesis

As one of the most prominent features in human GBM, pseudopalisading necrosis, the area of hypercellularity surrounding necrotic regions, and associated active vascular proliferation and tumor invasion are driven by hypoxia [32, 33]. Tumor cells reside in these regions have a high expression of HIF-1α and release VEGF, which is one of the most important regulators of angiogenesis and neovascularization (**Figure 1**). VEGF family members signal predominantly through the cognate RTKs, VEGFR-1, VEGFR-2, and VEGFR-3, in association with the co-receptors [34] via both hypoxia-dependent and hypoxia-independent mechanisms. Moreover, pseudopalisading necrosis regions protect glioma stem cells (GSC) in the region from therapeutic agents, and this facilitates the GSC niche to expand and contribute to tumor growth [35]. HIF-1α is a transcription factor that regulates the expression of a variety of genes

involved in glycolysis, angiogenesis, invasion and epithelial-mesenchymal transition (EMT), which are critical for tumor growth and progression, and likely cooperate and activate other aberrant RTK signaling pathways [36, 37]. We and other reports showed that, in response to hypoxia condition, significantly increased activity of EGFR, as well as its mutant protein EGFRvIII, which further promoted activation of convergent downstream signaling pathways including Ras/MAPK, PI3K/Akt, JAK2/STAT3, and NF-κB signaling, and enhanced malignant behaviors in GBM cells in vitro, and most likely to act in the same way in vivo [1, 38].

In addition to VEGF, supplementary proangiogenic factors including FGF, PDGF, placenta-like growth factor (PLGF), integrins, HGF/scatter factor, ephrins, angiopoietins (ANGPT), and interleukin-8 (IL-8), matrix-metalloproteinase (MMP)-2, MMP-9, collagen type I α1 (COLIA1), endothelial markers CD34, Tenascin-C, neuron-glial antigen 2 (NG-2) on pericytes, insulin-like growth factor (IGF), and EGF present in GBM [39–42]. Interestingly, many of these factors are RTK ligands and may bind to respective RTK on vascular endothelial cells or GBM cells, act in autocrine or paracrine manner to stimulate the events of neo-angiogenesis. Activation of these proangiogenic factors interacts with a number of signaling pathways include activation of Ras/Raf/MAPK [41, 42], PTEN/PI3K/AKT [40], PLC-γ/protein kinase C (PKC) [40], nitric oxide (NO) [43], PDGFB [44], and Notch1 [45]. GBMs are diagnosed at the advanced stages when they show hypoxia and leaky vasculatures [35]. The critical role that VEGF and these pro-angiogenic factors play in angiogenesis has rendered them appealing targets to exploit in cancer therapeutics [43].

3.2. RTK and ECM/integrin signaling

ECM/ integrins are key components mediating the dialog between cells and the microenvironment. Integrins are composed of two noncovalently associated α and β subunits, which are featured by a large extracellular domain, a short transmembrane domain and a small intracellular noncatalytic cytoplasmic tail [46]. These receptors play a role in the regulation of cell adhesion to ECM proteins or cell surface proteins [47]. Binding of ECM to integrins result in cell adhesion and activation of focal adhesion (FA)-associated signaling pathways [48] and thereafter cascades of intensive activation of downstream signaling that involved in cell proliferation and invasion [49]. Within FA complexes, further auto-phosphorylation of focal adhesion kinase (FAK) leads to its binding to SRC kinase and formation of activated FAK-SRC complexes. Consequently, FAK-SRC complexes activate cascades of downstream pathways including the Ras/Raf/MAPK, RAF/JNK, Rho/Rac/PAK and PI3K/Akt/mTOR [50]. Notably, FAK protein is overexpressed in many tumors including GBM, and its expression level is greatly correlated with poor clinical prognosis [51, 52].

ECM dysregulation is essential for establishing and maintaining a functional tumor microenvironment. ECM in GBM is stiffer and more cross-linked than that in the normal brain tissue, inducing abnormal cell behaviors such as aggressive cell invasion [35]. Dysfunction of ECM and its cognate receptor integrin may lead to aberrant signaling transduction pathways including Ras/Raf/MAPK, Raf/JNK, Rho/Rac/PAK and PI3K/Akt/mTOR, shaping a tumor microenvironment to promote tumor survival, angiogenesis, and invasion [31]. Importantly, many of these cellular signaling pathways are convergent with downstream signaling pathways of RTKs, implicating interaction and cross-talk of RTK- and ECM/integrin-mediated function in GBM

invasiveness and aggressiveness [53]. We demonstrated that hypoxia tumor microenvironment and ECM vitronectin could enhance tumor cell invasion and EGFRvIII activity via EGFRvIII and integrin β3 complex, emphasizing key roles of TME in tumor progression and metastasis [54]. Furthermore, as ECM may act as a reservoir for multiple growth factors such as VEGF, EGF, PDGF and TGF-β, release of these factors and their binding to their cognate receptors may also converge and further strengthen the activation of these signaling cascades, leading to uncontrolled cell behaviors in tumor growth and survival, angiogenesis, and invasion [55].

Knockout studies show the role of integrins in overactive GBM angiogenesis, which highly depends on VEGF and bFGF [56], αvβ3, αvβ5, β1, and αvβ8 notably play an important role during the process. For example, endothelial cells-expressed αvβ3/αvβ5 can provide survival signals and traction for invading cells, which are necessary to angiogenesis [57, 58]. αvβ3/αvβ5-associated neovascularization is respectively dependent on tumor cell-secreted bFGF/TNFα and VEGF and involved in a process leading to active interaction between tumor cells and endothelial cells [59]. Overexpression of αvβ3/αvβ5 in endothelial cells facilitates adhesive interactions with ECM proteins such as vitronectin, fibronectin, fibrinogen, osteopontin, etc. In cooperation with bFGF/VEGF, αvβ3/avβ5 also activates signaling pathways including FAK/ILK, PI3K/Akt, and SDF1-CXCR4 [60] that promote EC proliferation, survival, and migration [61], and initiation of tumor angiogenesis. Collectively, several key integrins such as αvβ3, αvβ5, β1, and αvβ8 appear to be potential targets in GBM to reduce tumor angiogenesis [62].

3.3. RTK-mediated immune suppression

GBM patients show marked intratumoral and systemic immunosuppression. The tumor microenvironment contains multiple immunosuppressive factors including TGFβ2, prostaglandin-E2, IL-10; and receptor molecules B7-H1, Fas-ligand, etc. [63]. The tumor is heavily infiltrated by microglia/macrophages, which can represent up to 30% of viable cells in the tumor mass, but lymphocytes infiltration is not common [64]. These monocytes/macrophages in the tumor environment interact with GBM cells and develop immunosuppressive myeloid-derived suppressor cells (MDSCs). Systemic immunosuppression in GBM patients shows that total T-cell counts are greatly decreased, especially CD4+ T cell counts [65]. Furthermore, T-cell function is markedly abnormal [66]. Besides other factors that may underlie T-cell dysfunction, increases in circulating cell populations such as regulatory T cells (Tregs) and MDSCs may be more important [67, 68].

Because of immunosuppressive features of GBM, in recent years, new therapies such as tumor vaccines and peptides are tested in preclinical and clinical studies [63]. The mutant protein EGFRvIII is a cancer specific antigen bearing a targetable epitope that is almost exclusively present in GBM [69]. Rindopepimut is composed of an EGFRvIII-specific peptide conjugated with an adjuvant protein KLH (keyhole limpet hemocyanin). The vaccination produced active anti-tumor response with significant survival benefit in GBM patients. Furthermore, the underlying immune response is not only effective regarding specific removal of EGFRvIII-positive GBM cells, but also the increase in the titer of anti-EGFRvIII sera in beneficial patients [70]. More importantly, phase II clinical trial with the vaccine confirmed these results [71], and the randomized phase III clinical study is ongoing [63]. EGFRvIII lacks the ligand binding domain

and is persistently activated, promoting tumor formation by activating aberrant signaling pathways, epigenetic mechanisms, and metabolic networks, and thus, is a promising cancer target [72]. Further with rindopepimut efficacy, chimeric antigen receptor (CAR) T cells transduced with humanized scFv against EGFRvIII were produced, and the studies are ongoing [73]. Other immunotherapy approaches that are tested or ongoing in GBM clinical studies include the administration of dendritic cells-based therapies; application of check-point inhibitor drugs, and adoptive cell therapy (ACT), etc.

EGFR also plays a protruding role in GBM cell invasion and aggressiveness [74]. It was previously showed that microglia cells stimulate GBM invasion via the EGFR signaling [75]. Coniglio et al. [76] demonstrated in vitro that microglia secreted EGF, which may activate EGFR and signaling pathways in GBM cells [77]. For example, EGFR or EGFRvIII may activate the STAT3 pathway [38], which is induced in various immune populations, and mediate immunosuppression potentiated by the GSCs [78]. Moreover, recent data implicated VEGF as a potent mediator of immunosuppression, again via GSC-associated mechanisms [79, 80]. A VEGF inhibitor, aflibercept, was applied in combination with an antitumor vaccine. Delayed tumor progression and survival extension were observed, which confirmed the efficacy of combining antiangiogenic and immunotherapy approaches, as well as the value of delineating tumor microenvironment [81]. Antiangiogenic therapy added to immunotherapeutic approaches toward glioma may show clinical benefits, among which the endogenous microenvironment or vaccine-induced inflammatory responses is importantly subsidiary to its effectiveness [82].

3.4. RTK, GSC and tumor metabolism

GSCs or glioma initiating cells (GICs) are preferentially located in perivascular and around necro/hypoxic zones where they closely react with the microenvironment and, thus, escape from apoptotic stimuli and preserve the capacity of self-renewal [83]. These interactions with microenvironment components, such as stromal cells or extracellular matrix (ECM) etc., seem important for GSC maintenance, possibly via metabolic and/or epigenetic modifications [83, 84]. Besides, GSCs may be protected from external factors via specific survival signals that they receive from the niche [85]. For instance, hypoxia induces VEGF expression, which promotes angiogenesis and supports the GSC tumor-initiating capacity [86, 87].

Besides VEGF/VERGFR signaling, HGF/Met signaling involves in regulating cell growth, motility and has a role in embryogenesis, degenerative disease and wound healing [88]. This RTK-mediated signaling also promotes the acquisition of stem-cell like properties in glioma cells and the formation and malignant progression of GBM [89, 90]; overexpressing of Met in vitro in glioma cells was highly clonogenic [88]. Met expression seems to be associated with genetic features with EGFR and the tumor suppressor PTEN inactivation, indicating cooperation among these RTK-mediated signaling in keeping GSC phenotype in glioma [91, 92]. A recent mouse study showed that EGFR inhibition induces increased c-Met expression and associated proliferation of GSCs expressing pluripotency TFs and displaying multi-lineage potential [89]. There is now the debate as to the long-term safety of anti-EGFR treatments, which may possibly induce MET-driven GSC populations [88, 89]. On the other hand, however, it implies the combination of targeting EGFRvIII and GSC as a new therapeutic approach.

Cancer development, progression, and response to treatment are greatly influenced by cancer cells' intracellular metabolism and the exogenous tumor environment [93]. The metabolic reprograming that cancer cells adapted to take up and utilize nutrients to drive tumor growth rigorously often relies on signaling and epigenetic/transcriptional networks induced by activated oncogenes (e.g., EGFR, RAS, MYC) and deactivated tumor suppressor proteins (e.g., TP53) [94, 95]. In primary GBM, the frequent genetic changes of key components of RTK/PI3K/Akt pathways, one of the three core signaling pathways that significantly altered in GBM [96], may result in constitutive activation of mechanistic target of rapamycin (mTOR) signaling [96, 97].

Recent studies showed cooperation between EGFRvIII signaling and c-Myc, the transcription factor and one of the master regulators of cancer metabolism [96], to reprogram cellular metabolism and promote tumor proliferation via activation of mTOR signaling, resulting in changes in intracellular nutrients levels [98–100]. Moreover, RTK- and Myc-dependent metabolic reprogramming maybe also involved in IDH1-mutant glioma malignant progression [101, 102]. Therefore, targeted therapies against Myc-dependent metabolism may be an effective therapy for patients with high-grade gliomas. Recent data indicated that extracellular nutrients such as glucose or acetate were required to maintain EGFRvIII signaling via activating mTORC2 [96], leading to GBM resistance to molecularly targeted therapies [103]. Nonetheless, the intricate interactions between the oncogenic signaling and cancer metabolism have only been recently revealed, and metabolism in primary GBM is dominantly regulated hypoxia and RTK-dependent c-Myc upregulation to modify cancer metabolome and cause resistance to therapies [93]. Future studies are needed to govern regulations on genetic and epigenetic alterations, oncogenic signaling and cancer metabolic reprograming, and translate these insights into more effective treatments for GBM patients.

4. Lessons from RTK-targeted therapies

Since the phase III trials of Temozolomide in 2005, there have been few successes regarding treatment for patients with malignant glioma [82]. RTKs, which are the most commonly amplified and mutated genes in cancers, are the key targets in cancer research including malignant glioma. Up to date, three RTKs and their family members present the major druggable targets, including EGFR and EGFRvIII, VEGFR and PDGFR family.

4.1. Experience with RTK-targeted therapies in GBM

4.1.1. EGFR family and EGFRvIII

Small molecular weight kinase inhibitors include gefitinib (EGFR) and erlotinib (EGFR and EGFRvIII), and these two irreversible inhibitors, unfortunately, have achieved limited success either as a single agent or as combination therapies in numerous Phase I and II trials in patients with newly diagnosed or recurrent GBM [104–107]. The resistance may be driven by a subset of EGFR mutations, activation of alternate signaling pathways and suppression of EGFRvIII on extrachromosomal DNA, etc. [72]. Besides, irreversible inhibitors currently in clinical trials including lapatinib (EGFR, ErbB-2), AEE788 (EGFR, VEGFR), and dacomitinib

(EGFR, HER2, HER4), alone or in combination with other agents, still attained minimal to moderate anti-tumor response in newly diagnosed GBM or recurrent patients [108, 109].

Monoclonal antibodies (mAbs) targeted against both wild-type EGFR and EGFRvIII have also been developed including cetuximab, which showed only minimal anti-tumor effect as a single agent in Phase I/II trials [110], but the drug showed chemosensitizing and radiosensitizing effect and may achieve better effect when combined with TMZ and radiotherapy [111]. Other anti-EGFR antibodies include panitumumab and nimotuzumab. Nimotuzumab, a humanized mAb against EGFR, has shown promising efficacy with significantly higher mean and median survival time in GBM patients in Phase I/II trials via its inhibition on tumor growth and angiogenesis; the antibody drug also shows least cutaneous toxicity [82]. Furthermore, targeting at EGFRvIII which acts as a GBM-specific antigen, rindopepimut is a promising peptide vaccine and has shown its effectiveness and induced strong and specific immune response in Phase II clinical trials [70, 112]. The vaccine is currently investigated in Phase II/III trials in newly diagnosed GBM patients alone, in combination treatment with other agents or standard treatment protocol in recurrent patients [82].

4.1.2. VEGFR family

VEGFR is the most potent stimulator in angiogenesis, mainly including VEGFR1, VEGFR2, and VEGFR3. Several VEGFR inhibitors have been developed and applied in preclinical and clinical studies in GBM.

Cediranib (AZD2171) is a pan-VEGFR RTK inhibitor; in addition, it inhibits activity of RTKs including c-Kit, PDGFRA and PDGFRB. In Phase II trials, cediranib treatment quickly induces tumor vessel normalization and edema reduction which were related with the progression-free survival (PFS) in GBM patients [113]. The treatment with cediranib is associated with improved overall survival (OS) only in newly diagnosed GBM patients [114]. Various other VEGFR inhibitors including aflibercept, BIBF 1120, pazopanib, AMG 386 (trebananib), Vandetanib are tested in combination with other drugs in their Phase I/II trials [115]. Among these, aflibercept inhibits both VEGF and placental growth factor (PGF), and acts as a decoy receptor dubbed VEGF trap, yet shows limited success in Phase II trials for recurrent GBM patients [116]. The mAb against VEGF, bevacizumab (Avastin®) is currently used in patients with GBM, mostly in combination with other treatment or drugs. When used in combination with irinotecan, a cytotoxic topoisomerase I inhibitor, the treatment resulted in objective radiographic responses and improvement in PFS [117]. Since bevacizumab was approved by FDA in 2004, over 60 countries apply it for the treatment of progressive disease including the USA and Japan [118–120]. Two completed Phase III trials indicated an improved PFS but not OS of newly diagnosed GBM patients with combination of bevacizumab with the standard protocol (TMZ and RT), yet showed inconsistent results on patient performance status during the treatment [121, 122].

Research data indicated that resistance to VEGF/VEGFR targeted inhibition in GBM may activate other angiogenic factors, such as FGF and PDGF, and thus, promote alternate signaling for neovascularization [123]. Moreover, the treatment-induced HGF/c-Met activation may contribute to robust invasion in the resistant GBMs [124]. Combinational targeting strategies in a good timing with VEGFR-targeted agents warrant further investigations.

4.1.3. PDGFR family

A number of PDGFR inhibitors with multiple targets are developed and tested, including imatinib mesylate (PDGFR, c-KIT, BCR-ABL), sunitinib (PDGFR, VEGFR, c-KIT), sorafenib (PDGFR, VEGFR, RAF), tandutinib (PDGFR, FLT3, c-KIT), vatalanib (PDGFR, VEGFR, c-KIT), pazopanib (PDGFR, c-KIT, EGFR) or dasatinib (PDGFRB, Src, BCR/Abl, c-KIT, ephrin A2) [125]. Among these, Imatinib (Gleevec®) is already used for the first-line treatment of myeloid malignancies and gastrointestinal stromal tumors; however, as a single agent, it shows minimal efficacy in GBMs. Previously, a combination of imatinib and hydroxyurea, a cytotoxic agent that inhibits DNA synthesis, showed a 20% response rate in progressive chemo- and radio-refractory GBM patients [125]; however, similar combination treatment achieved minimal response in recent studies [126]. Other combinations of imatinib with cytotoxic agents, or other kinase inhibitors, have been tested at the preclinical levels and clinical studies [127, 128]. Thus far, this class of targeted agents only achieved minimal anti-tumor activity either alone or in combination with other therapies [82].

4.2. Mechanisms of resistance to RTK-targeted therapy

RTK targeted therapeutic strategies in cancer came into cancer practice since 2001, when FDA promptly approved imatinib (Gleevec®) as a first-line targeted agent for the treatment of patients with chronic myeloid leukemia (CML). In 2004, FDA approved bevacizumab (Avastin®) as a combination agent with standard chemotherapy to treat progressive disease such as metastatic lung cancer [129]. Thus far, however, RTK-targeted treatment strategies have achieved only moderate anti-tumor activity in patients with GBM [1, 35, 82]. Two RTK family as major targets including EGFR family and EGFRvIII, and anti-angiogenesis therapy against VEGF/VEGFR family are applied in newly diagnosed or recurrent GBM [82]. The experience with EGFR RTK inhibitors in GBM proved that, even if EGFR itself gets efficiently dephosphorylated in tumors, the treatment-induced EGFR-independent regulatory circuits may promote alternate activation of downstream signaling and render clinically ineffective [1]. Similarly, in the case of VEGFR-targeted therapy, alternative activation of other pro-angiogenic factors, such as FGF, PDGF, HGF, ANGPT2 and IL-8 et al. may still activate downstream effectors on converged signaling pathways [117–122]. Moreover, RTK heterogeneity and cooperation between RTKs, as well as secondary activation of downstream signaling pathways, may compensate for the loss of the targeted RTK [2]. For example, EGFRvIII transcriptionally inhibits PDGFRβ in tumor cells. EGFR TKIs reduces such inhibition, enabling tumor cells to switch their dependence to PDGFRβ for growth and survival [1].

The inherent link between RTK and TME greatly contribute to the resistance or even failure with the RTK-targeted therapy and combinational therapies [31, 35, 82]. Treatment with the VEGFR2 inhibitor vatalanib only achieved transient benefits on reduction of tumor vascular volume but induced hypoxia and was related to the increased expression of several pro-angiogenic cytokines and chemokines such as VEGF, SDF-1, HIF-1α, FGF, Ephrin, and their receptors including VEGFR2, VEGFR3, and EGFR, which promoted aggressive tumor invasion [130] and alternative pathway of neovascularization [131, 132]. Other RTK inhibitors such as cediranib and sunitinib have been associated with higher toxicities in clinical trials [133–137]. Recent data suggest that tumors have several distinct mechanisms of

neovascularization including vascular mimicry (VM) [138]. VM is identified as tumor cells, most likely GSCs, transdifferentiate into endothelial cells and form neovascular structures to irrigate the hypoxic tumors for both nutrients and active metabolism [139, 140]. GSCs also transdifferentiate into pericytes to maintain VM [141]. Thus, VM is one of the key tumor-inherent mechanisms to drive the resistance to anti-angiogenesis therapy in GBM [142–144]. Indeed, resistance to RTK-targeted and combination therapies is associated with accumulation of GSC as well as immune suppression. Achyut et al. reported that vatalanib treatment increased the number of CD68+ myeloid cells and the CD133+, CD34+, and Tie2+ endothelial cell signatures in a mouse model of GBM [145]. The enhanced myeloid cell infiltration in the TME following therapeutic resistance was associated with the activation of the CSF1–CSF1R pathway, which results in increased number of tumor-associated macrophages (TAM) within dynamic TME [146, 147].

Collectively, the mechanisms of resistance to RTK-targeted therapy include (1) intratumoral heterogenicity of RTKs, that is, cooperation of various RTKs and their downstream signaling pathways; (2) intertumoral heterogenicity of RTK expression and activity within TME; (3) the treatment-induced shaping and adaption of TME including secondary hypoxia, accumulation of GSC and immune suppression [77]. These mechanisms may cause from ineffectiveness to treatment failure, or even clinical toxicity, leading to GBM recurrence. Moreover, during RTK-targeted treatment, most clinical studies actually lack sufficient information regarding the measurement on intratumoral drug levels, target engagement and the degree of inhibition on the targeted RTK in real time [82]. Nonetheless, design of further combination therapies should consider such information, in addition to monitoring the tumor dynamic profiles, and treat the patients according to the corresponsive patterns in disease progression. Therefore, understanding the biology of CNS tumors and influence of TME on tumor progression is becoming increasingly important for developing new therapeutic strategies for this deadly disease.

5. Conclusion and future perspectives

Not to mention intertumoral heterogeneity of the RTK expression, intratumoral heterogeneity, in particular the heterogeneity of amplified and mutated RTKs, presents a serious challenge to design successful single agent and/or combination therapies for patients with GBM. Thus far, clinical trials with small molecules kinase inhibitors still did not change the clinical practice in human malignant glioma [148]. GBM is one of the most challenging malignancies as featured with its infiltrating nature, recurrent tendencies and poor response to any treatment modalities, besides the intertumoral and intratumoral heterogeneity [31, 82]. The major treatment challenges contain aberrant signaling pathways, hypoxic microenvironment, phenotypic and genetic heterogeneity, GSCs and the blood-brain barrier (BBB) [1, 35, 82]. Nonetheless, aberrant RTK mutation and associated signaling pathways are hallmarks of primary GBM. As we show in this chapter, the functional interaction between RTKs and TME in GBM significantly promotes more aggressive tumor invasion, neovascularization and hypoxia, increases the number of GSCs, and adapts tumor metabolism. Thus, considering the importance of the TME

in modulating cellular, molecular and epigenetic changes in a tumor cell, we propose that immunotherapy, especially vaccine-based treatment, targeting hypoxic cancer cells or HIF, and GSC-based therapies may be among the most promising strategies in GBM, in which reasonable and well-designed RTK-targeted therapy may at least partially contribute to the treatment success [31]. Dynamic treatment data measurement and personalized medicine with new imaging modalities (PET) using hypoxia radiotracers are key to delineating the hypoxic tumor regions, clinical tissue biopsy profile monitoring, and well-adjusted drug delivery systems may be rigorously applied to ensure therapeutic efficacy in GBM.

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