

# Chapter 8

## Receptor Tyrosine Kinases: Principles and Functions in Glioma Invasion

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**Abstract** Protein tyrosine kinases are enzymes that are capable of adding a phosphate group to specific tyrosines on target proteins. A receptor tyrosine kinase (RTK) is a tyrosine kinase located at the cellular membrane and is activated by binding of a ligand via its extracellular domain. Protein phosphorylation by kinases is an important mechanism for communicating signals within a cell and regulating cellular activity; furthermore, this mechanism functions as an “on” or “off” switch in many cellular functions. Ninety unique tyrosine kinase genes, including 58 RTKs, were identified in the human genome; the products of these genes regulate cellular proliferation, survival, differentiation, function, and motility. Tyrosine kinases play a critical role in the development and progression of many types of cancer, in addition to their roles as key regulators of normal cellular processes. Recent studies have revealed that RTKs such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), c-Met, Tie, Axl, discoidin domain receptor 1 (DDR1), and erythropoietin-producing human hepatocellular carcinoma (Eph) play a major role in glioma invasion. Herein, we summarize recent advances in understanding the role of RTKs in glioma pathobiology, especially the invasive phenotype, and present the perspective that RTKs are a potential target of glioma therapy.

**Keywords** Glioma • Glioblastoma • Invasion • Tyrosine kinase receptor • EGFR • PDGFR • c-Met • Tie • Axl • DDR1 • Eph • TrkA • Cross-talk • Tyrosine kinase inhibitor • Clinical trial

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## Abbreviations

Ang	Angiopoietin
BBB	Blood brain barrier
CTGF	Connective tissue growth factor
DDR1	Discoidin domain receptor 1
EC	Endothelial cell
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
Eph	Erythropoietin-producing human hepatocellular carcinoma
ERK	Extracellular signal-regulated kinase
FAK	Focal adhesion kinase
FGFR	Fibroblast growth factor receptor
Gas6	Growth arrest-specific gene 6
GBM	Glioblastoma multiforme
GPI	Glycosylphosphatidyl-inositol
HB-EGF	Heparin-binding EGF-like growth factor
HGF	Hepatocyte growth factor
HIF	Hypoxia inducible factor
IDH1	Isocitrate dehydrogenase-1
JAK	Janus kinase
MAPK	Mitogen-activated protein kinase
MEK	MAPK kinase
MMP	Matrix metalloproteinase
mAb	Monoclonal antibody
MT1-MMP	Membrane-type 1-MMP
NGF	Nerve growth factor
NF- $\kappa$ B	Nuclear factor-kappa B
OS	Overall survival
PDGFR	Platelet derived growth factor receptor
PFS	Progression-free survival
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PLC	Phospholipase C
PTEN	Phosphatase and tensin homolog deleted from chromosome 10
PTK	Protein tyrosine kinase
RTK	Receptor tyrosine kinase
STAT	Signal transducer and activator of transcription
TAMR	A member of the Tyro3, Axl, and Mer family of receptor tyrosine kinase
TCGA	The Cancer Genome Atlas
TGF- $\alpha$	Transforming growth factor alpha
TKI	Tyrosine kinase inhibitor
TMZ	Temozolomide
TrkA	Neurotrophic tyrosine kinase receptor type 1
uPA	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor

## 8.1 Introduction

Glioblastoma (GBM) is an extremely aggressive, highly vascularized, infiltrative tumor with a median survival duration of 12–15 months after initial diagnosis (Nagane 2011). GBMs invade the surrounding brain tissue, making complete surgical excision highly improbable. Despite current therapeutic strategies, these tumors almost universally recur because of the invading GBM cells left after excision and are associated with a poor survival rate (Nakada et al. 2007).

Currently, numerous studies are attempting to decipher the molecular mechanism of invasion and to better understand the molecular mechanisms responsible for invasion processes (Chuang et al. 2004; Salhia et al. 2005; Nakada et al. 2007; Onishi et al. 2011). Among the many molecules that were reported as invasion promoters, members of the protein tyrosine kinase (PTK) family play a major role in modulating invasion (Nakada et al. 2007). The human protein kinase genome contains 518 protein kinase genes, including PTK genes that encode transmembrane receptor tyrosine kinases (RTKs) and soluble cytoplasmic tyrosine kinases that are also known as non-RTKs (Manning et al. 2002). More than 58 mammalian RTKs and 37 non-RTKs have been identified. RTKs contain an intracellular catalytic PTK domain and regulatory sequences, a transmembrane domain, and an extracellular ligand-binding domain. RTKs modulate a wide range of cellular events, including proliferation, migration, metabolism, differentiation, and apoptosis, under physiological as well as pathological conditions (Schlessinger 2000). The phosphorylation of tyrosine residues in RTKs is essential for maintaining cellular homeostasis and modulating gene expression in various intercellular and intracellular signaling pathways. Because the complex signaling network triggered by RTKs eventually leads to either activation or repression of various gene subsets, RTKs regulate intercellular communication and control cell proliferation, mitogenesis, survival, differentiation, motility, and metabolism (Schlessinger 2000). The individual cellular consequences of RTK activation are complex and depend on the cell type and the activated signal transduction pathway.

Several lines of experimental evidence have revealed that aberrant RTK activation frequently occurs during glioma initiation and progression and that these tumorigenic cascades may cooperate through multiple signaling cross-talks in the malignant transformation of cells, treatment resistance, and disease relapse (Table 8.1). Because GBMs actively synthesize a substantial variety of RTKs that contribute to invasion, a systematic approach to inhibiting RTKs is being undertaken as a treatment adjunct.

**Table 8.1** RTK associated with glioma invasion

RTK	Ligand	Expression in GBM	Amplification in GBM	Downstream signaling
EGFR	EGF	High	40 %	PI3K/Akt, Ras/MAPK, FAK, MMP-2
PDGFR	PDGF	High	11 %	PI3K/Akt, Ras/MAPK, JAK/STAT
ERBB2	Neuregulin-1	High	8 %	PI3K/Akt, Ras/MAPK, PLC- $\gamma$ , JAK/STAT, MT-MMP, FAK
c-Met	HGF	High	20 %	PI3K/Akt, Ras/MAPK
Tie	Ang	High	Not reported	MMP-2
Axl	Gas6	High	Not reported	PI3K/Akt
DDR1	Collagen	High	Not reported	MMP-2
Eph	Ephrin	High or low	Not reported	R-Ras, Rac1
Trk	NGF, CTGF	High or low	Not reported	NF-kB, ZEB-1

**Designations:**

Ang, angiopoietin; CTGF, connective tissue growth factor; DDR1, discoidin domain receptor 1; EGFR, epidermal growth factor receptor; Eph, erythropoietin-producing human hepatocellular carcinoma; FAK, focal adhesion kinase; Gas6, growth arrest–specific gene 6; HGF, hepatocyte growth factor; MAPK, Mitogen-activated protein kinase; PDGFR, platelet derived growth factor receptor; PI3K, phosphatidylinositol-3-kinase

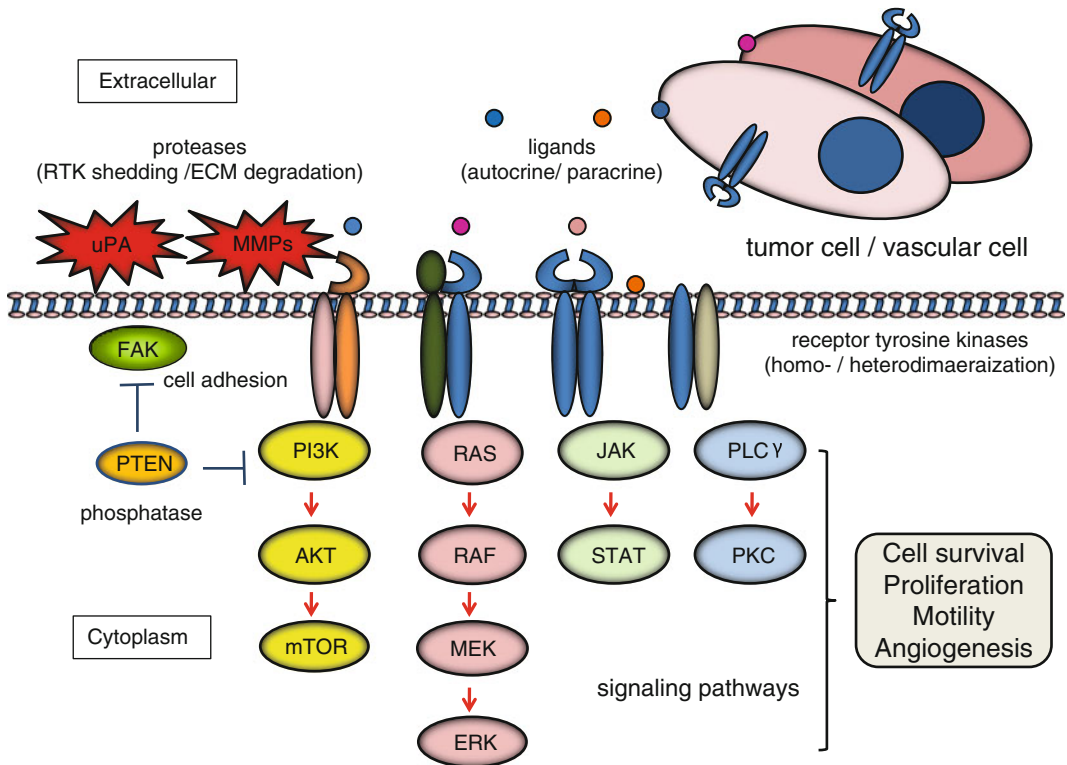
In this chapter, the most recent advancements in the structural and functional characterization of invasion signal transduction elements of the RTK signaling network and the molecular mechanisms involved in glioma invasion are described. We provide an overview of this field, highlighting areas with the strongest research evidence for the translational potential of the use of tyrosine kinase inhibitors (TKIs).

## 8.2 EGFR/EGF

Epidermal growth factor receptor (EGFR) belongs to a large family of cell surface receptors with intrinsic protein tyrosine kinase activity. The EGFR family comprises four members designated EGFR (also known as ErbB1/HER1), ErbB2 (HER2/Neu), ErbB3 (HER3), and ErbB4 (HER4) (Jorissen et al. 2003). Six ligands are known to activate EGFR: EGF, transforming growth factor alpha (TGF- $\alpha$ ), amphiregulin, betacellulin, heparin-binding EGF-like growth factor (HB-EGF), and epiregulin (Bogdan and Klambt 2001; Normanno et al. 2006). These ligands are secreted by glioma cells as well as by tumor microenvironmental cells such as microglia and reactive astrocytes (Hoelzinger et al. 2007). Upon binding of extracellular ligands, EGFR undergoes dimerization, resulting in trans-autophosphorylation of its cytoplasmic domain. EGFR can pair with another EGFR to form an active homodimer or with another member of the EGFR family to create a heterodimer (Yarden and Sliwkowski 2001). For example, EGFR can easily form heterodimers with ErbB2, which has a reduced internalization capacity compared with that of EGFR and thus prolongs EGFR signaling (Jones et al. 2006) (Fig. 8.1).

EGFR and its ligands are often over-expressed in human carcinomas. In GBM, EGFR gene amplification is the most frequent RTK alteration (approximately 40 %) (Libermann et al. 1985). EGFR overexpression and/or gene alteration is frequently observed in primary (*de novo*) GBM, which develops rapidly, has a short clinical history, and does not show evidence of less malignant precursor lesions (Ekstrand et al. 1992; Ohgaki et al. 2004; Wong et al. 1987). Genomic analysis by The Cancer Genome Atlas (TCGA) network revealed that *EGFR* aberration is related to the classical subtype of GBM (Network 2008). Amplification of the *EGFR* gene is also associated with structural alterations and the most common of these is called EGFR variant III (EGFRvIII). EGFRvIII is a mutant with an in-frame deletion of exons 2–7 from the extracellular region and can transmit constitutive growth signaling in a ligand-independent manner (Ekstrand et al. 1992; Yamazaki et al. 1988). EGFRvIII expression in glioma cells stimulates expression of TGF- $\alpha$  and HB-EGF, suggesting that EGFRvIII plays a role in generating an autocrine loop with wild-type EGFR expression (Ramnarain et al. 2006). Genetic alterations that affect EGFR signaling result in the activation of several downstream pathways such as the phosphatidylinositol 3-kinase (PI3K)/Akt and Ras/Raf/MEK (MAPK kinase)/MAPK (mitogen-activated protein kinase) pathways, which mediate cell proliferation, survival, and mobility (Kita et al. 2007; Ohgaki and Kleihues 2009). The PI3K/Akt pathway is negatively regulated by the tumor suppressor phosphatase and tensin homolog deleted from chromosome 10 (PTEN), which is mutated in 20–40 % of GBMs and is a hallmark of this disease (Ohgaki et al. 2004). PTEN dephosphorylates focal adhesion kinase (FAK), which is a key molecule for cell interaction with the extracellular matrix (ECM) (Gu et al. 1999; Kita et al. 2001; Tamura et al. 1998). Dephosphorylated FAK interferes with EGFR vIII-mediated glioma cell invasion, indicating that the EGFR-PTEN-FAK interaction plays an important role in glioma invasion (Cai et al. 2005) (Fig. 8.1).

Several reports have showed that GBM patients with EGFR overexpression or mutation have shorter survival, suggesting that EGFR alterations are associated with highly aggressive GBMs (Barker et al. 2001; Feldkamp et al. 1999; Shinojima et al. 2003). One report indicated that coexpression of EGFRvIII and PTEN was a positive indicator of responsiveness to EGFR inhibitors (e.g., gefitinib and erlotinib) in patients with GBM (Mellinghoff et al. 2005).



**Fig. 8.1** Cell signaling pathways induced by receptors tyrosine kinase (RTK). Homo-/heterodimerization of RTKs are caused by their ligands in autocrine or paracrine fashion. The dimerized receptors can initiate signal transduction cascades involved in cell survival, proliferation, motility and angiogenesis and so on, e.g.: phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR; RAS/RAF/mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK); Janus kinase (JAK)/signal transducer and activator of transcription (STAT); and phospholipase C $\gamma$  (PLC) $\gamma$ /protein kinase C (PKC). Examples of cross-talk between RTKs' signaling and proteins associated with cell invasion (e.g. urokinase-type plasminogen activator (uPA), matrix metalloproteinase (MMP)s, focal adhesion kinase (FAK) and phosphatase and tensin homolog deleted from chromosome 10 (PTEN)) are demonstrated. Red arrows and blue bars indicate activation and suppression, respectively (ECM, extracellular matrix)

### 8.3 PDGFR/PDGF

Following EGFR/EGF signaling, aberrant platelet-derived growth factor receptor (PDGFR)/PDGF signaling is one of the hallmarks of GBM biology. Overexpression of PDGFR subtypes  $\alpha$  and  $\beta$  and PDGF ligands A–D has been observed in glial tumors of all grades and is possibly associated with malignant progression (Fleming et al. 1992; Guha et al. 1995; Hermanson et al. 1992; Lokker et al. 2002; Nister et al. 1988; Ozawa et al. 2010). An experimental study revealed that glioma-like tumors can be induced after overproduction of PDGFB in the mouse brain (Uhrbom et al. 1998). Histochemical studies revealed that PDGFR $\alpha$  and PDGFA are expressed in glioma cells, whereas PDGFR $\beta$  and PDGFB have been found in the surrounding endothelial cells (ECs) (Hermanson et al. 1992; Plate et al. 1992). The expression of this receptor in blood vessels suggests that paracrine activation is also possible with respect to tumor cell migration and colony formation (Hoelzinger et al. 2007; Shih and Holland 2006). Although the expression of PDGFC and D ligands has also been demonstrated in gliomas, the clinical and biological significance of their expression has not been determined (Lokker et al. 2002). Ligand-receptor co-expressions in tumor cells allow for both autocrine and paracrine

forms of activation. Some reports suggest that PDGFR/PDGF signaling through this autocrine/paracrine pair results in increased GBM cell motility *in vivo* (Cattaneo et al. 2006; Natarajan et al. 2006). Furthermore, these autocrine and/or paracrine loops can stimulate downstream signal transduction pathways including Ras/MAPK, PI3K/Akt and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and have pivotal roles in proliferation, differentiation, survival, and invasion (Blume-Jensen and Hunter 2001; Khoshyomn et al. 1999; Valius and Kazlauskas 1993) (Fig. 8.1).

The association between PDGFR expression and the prognosis of glioma patients is controversial. PDGFR $\alpha$  expression in low-grade gliomas was reportedly associated with both poor (Varela et al. 2004) and favorable (Ribom et al. 2002) prognosis. Furthermore, PDGFA expression, but not PDGFR $\alpha$  expression, was useful in predicting tumor grade in oligodendrogliomas (Majumdar et al. 2009). PDGFR $\alpha$  expression was not associated with survival in a series of high-grade pediatric gliomas and GBMs (Liang et al. 2008; Martinho et al. 2009). However, the absence of PDGFA expression was significantly associated with poor prognosis in patients with glioma (Martinho et al. 2009). PDGFR and ligand overexpression tend to be associated with loss of the TP53 tumor suppressor, which is characteristic of secondary GBMs that develop from less malignant precursors (Ohgaki and Kleihues 2009). According to the TCGA consortium, *PDGFRA* amplification (11 %) and isocitrate dehydrogenase-1 (*IDH1*) mutation are hallmarks of a proneural subtype of GBM, suggesting an association between this subtype and secondary GBM (Network 2008; Verhaak et al. 2010). Another study demonstrated that *PDGFRA* amplification was observed in 21 % of gliomas, although *PDGFRA*-activating mutations were not found (Martinho et al. 2009).

## 8.4 ERBB2

ErbB2 (HER2/Neu) belongs to the EGFR receptor family that contains the other three members: EGFR, ErbB3, and ErbB4 (see EGFR/EGF section). Although the intracellular tyrosine kinase domain of the EGFR family is highly conserved, none of the EGFR family ligands bind ErbB2 because of its extracellular region structure. Thus, the role of ErbB2 depends on the patterns of dimerization within the family (Normanno et al. 2006). For example, among all possible ErbB2-containing heterodimeric receptor complexes, the most potent signaling module in terms of cell proliferation and *in vitro* transformation is the ErbB2/ErbB3 heterodimer, even though ErbB3 lacks kinase activity (Citri et al. 2003). These homo- or heterodimers have various potencies for the induction of signaling pathways such as the Ras/Raf/MEK/MAPK pathway for proliferation, the PI3K/Akt pathway for survival (Ben-Levy et al. 1994; Prigent and Gullick 1994), the phospholipase C (PLC)- $\gamma$  pathway for cell migration and invasion (Khoshyomn et al. 1999), and the STAT pathway for cell cycle regulation (Gao et al. 2010) (Fig. 8.1).

Forced ErbB2 expression can transform cells into the invasive phenotype in association with expression of membrane-type 1 (MT1) matrix metalloproteinase (MMP), which is a key enzyme in glioma invasion (Miyamori et al. 2000; Nakada et al. 2001; Sato et al. 1994). In addition, post-transcriptional shedding of cell surface ErbB2 has been reported to be processed by proteases that can degrade the ECM (e.g., MMP2, MMP9, MMP13, and uPA) (Gondi et al. 2009; Spencer et al. 2000; Yong et al. 2010), indicating a strong association between ErbB2 expression and glioma invasion.

Although ErbB2 protein expression is mainly seen in high-grade gliomas (Andersson et al. 2004; Engelhard et al. 1995), evidence for the prognostic value of ErbB2 expression levels in GBMs is presently sparse (Gulati et al. 2010; Haapasalo et al. 1996; Hiesiger et al. 1993; Mineo et al. 2007; Schwachheimer et al. 1994). On the other hand, both ErbB2 and ErbB4 expression levels have been shown to predict prognosis in childhood medulloblastoma and ependymoma (Gilbertson et al. 1997; 2002). Although few reports have described mutation of the *ErbB2* gene (Stephens et al. 2004), a recent study of the TCGA consortium revealed that mutation was observed in 7 of 91 (8 %) GBMs (Network 2008; Verhaak et al. 2010).

Furthermore, ErbB2 overexpression has been well described in human breast cancers (20–30 %), which correlates with more aggressive tumors and a poorer prognosis (Hortobagyi 2005).

## 8.5 c-Met/HGF

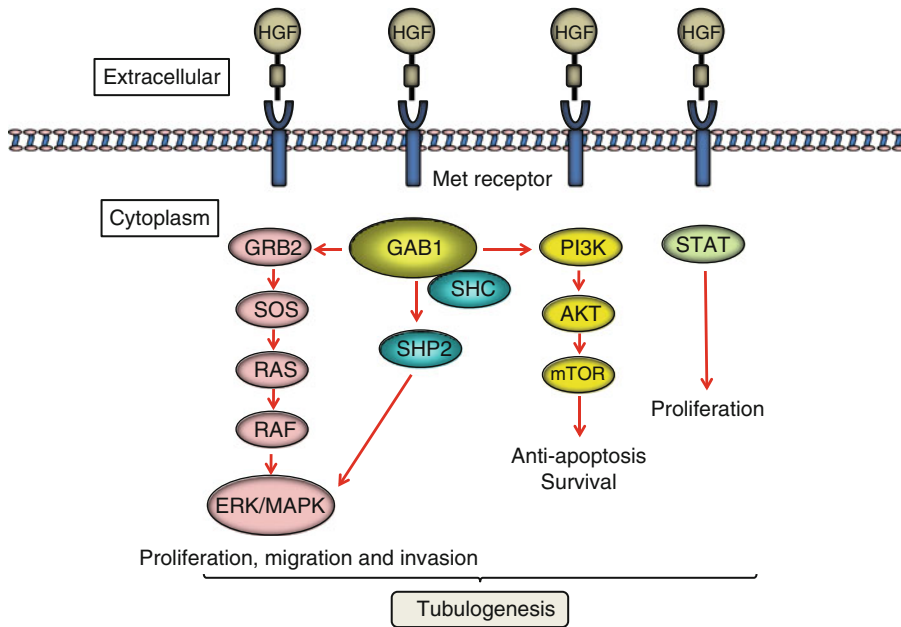
Hepatocyte growth factor (HGF) was originally identified as a polypeptide growth factor for hepatocytes and is believed to play an important role in liver regeneration (Yamada et al. 1994). HGF functions as a mitogen for a variety of cell types and as a morphogen and motogen for some epithelial cells that express its receptor (Moriyama et al. 1995). The receptor protein for HGF, c-Met, is encoded by the *c-met* proto-oncogene, which has tyrosine kinase activity and was originally described as an activated oncogene in a human osteosarcoma cell line (Cooper et al. 1984). Recently, attention has been focused on the role of the HGF/c-Met system because of its multiple biological activities including motility, proliferation, survival, and morphogenesis.

Under normal conditions, HGF-induced c-Met activation is tightly regulated by paracrine ligand delivery, ligand activation at the target cell surface, and ligand activated receptor internalization and degradation (Cecchi et al. 2010). Despite its functions under normal conditions, HGF/c-Met signaling contributes to oncogenesis and tumor progression in several cancers and promotes aggressive cellular invasiveness that is strongly linked to tumor metastasis (Rosario and Birchmeier 2003; Zhang and Vande Woude 2003).

The HGF/c-Met pathway has been implicated in a wide variety of human malignancies. Overexpression of HGF and/or c-Met is frequently observed and amplification of the *c-met* gene has been reported in several tumor types (Burgess et al. 2006). Several studies have described the expression of HGF and c-met as well as HGF activator mRNAs in glioma cell lines and tissues, particularly GBM (Koochekpour et al. 1997; Moriyama et al. 1995). On the other hand, the expression of HGF and c-Met was low or barely detectable in low-grade astrocytoma and c-Met immunoreactivity was correlated with the histological grade of the tumor.

A few studies suggested that HGF and c-Met are expressed in human gliomas and that expression levels correlated with tumor grade (Moriyama et al. 1998b). Likewise, HGF expression was significantly higher in high-grade tumors than in low-grade tumors based on HGF content detection in samples of different clinical grades (Abounader and Latterra 2005; Lamszus et al. 1999). c-Met receptor expression has also been detected in malignant brain tumors including all gliomas, medulloblastomas, ependymomas, and schwannomas (Koochekpour et al. 1997; Moriyama et al. 1998b). The results of an overexpression study provided sufficient evidence implicating the HGF/c-Met pathway in brain tumorigenesis and malignant progression; this study demonstrated that HGF/c-Met plays important and critical roles in brain tumor formation and growth.

The HGF/c-Met system in vascular ECs works as signal transduction molecules/pathways in gliomas that mediate neovascularization. Numerous *in vivo* and *in vitro* studies have indicated that HGF and c-Met are expressed and functional in neuromicrovascular and brain tumor vascular cells. The HGF/c-Met system is highly activated in cultured neural microvascular ECs. In gliomas, HGF stimulates the proliferation of neuromicrovascular ECs by paracrine and autocrine mechanisms. Other studies demonstrated that HGF-dependent interactions between glioma cells, and between glioma cells and the endothelium, can contribute to the heterogeneous proliferative and angiogenic phenotypes of malignant gliomas *in vivo* (Abounader and Latterra 2005). Moriyama et al. explored the effect of HGF on vascular endothelial growth factor (VEGF) expression in c-Met-positive human glioma cell lines and their results suggest that HGF can act as an indirect angiogenic factor through autocrine induction of VEGF expression and secretion in malignant gliomas in addition to its direct angiogenic activities (Moriyama et al. 1998a). Taken together, these *in vitro* and *in vivo* findings suggest the multifunctional and multilevel involvement of the HGF/c-Met pathway in brain tumor angiogenesis as well as brain tumor growth.



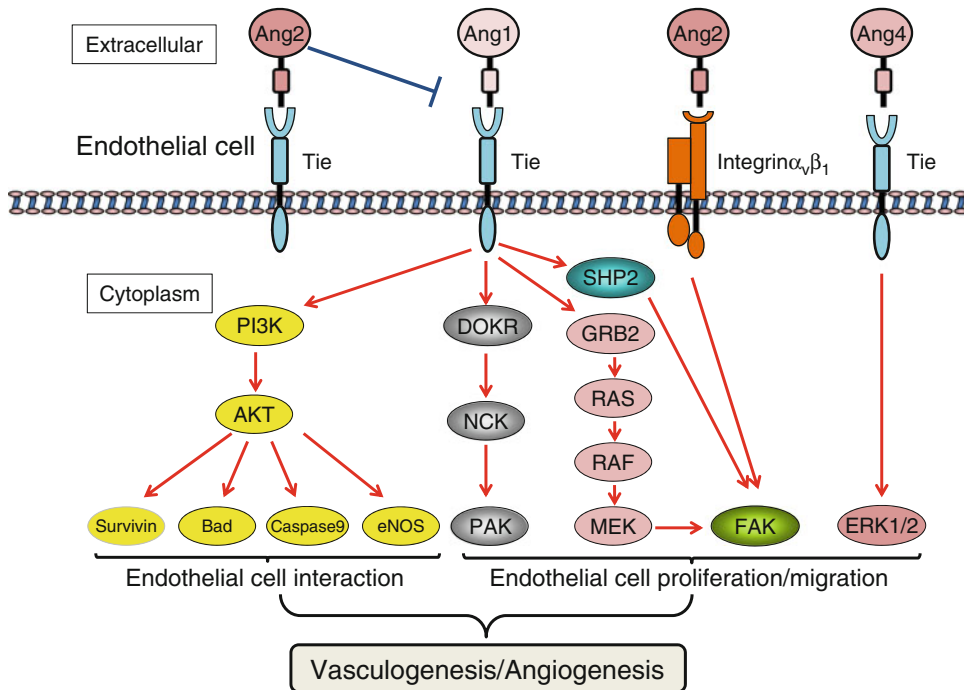
**Fig. 8.2** Major signaling pathways by the receptor tyrosine kinase c-Met. Growth factor receptor-bound protein 2 (GRB2), Grb2-associated adaptor protein 1 (GAB1), hepatocyte growth factor (HGF), phosphatidylinositol 3-kinase (PI3K), son of sevenless (SOS), rat sarcoma oncogene homolog (RAS), extracellular receptor kinase (ERK), mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT), SRC, SRC homology protein tyrosine phosphatase 2 (SHP2), SRC homology domain c-terminal adaptor homolog (SHC), mammalian target of rapamycin (mTOR)

Activation of the HGF/c-Met axis promotes proliferation and survival through a variety of downstream effectors including Gab1, Grb2, and PI3K (Abounader and Laterra 2005; Ponzetto et al. 1994). Following activation of the HGF/c-Met pathway, the RAS-MAPK signaling pathway plays an essential role in morphogenesis. The activation of c-Met prevents apoptosis through activation of PI3K and subsequent Akt activation. Cross-talk between the PI3K/Akt and the RAS-MAPK pathways has been implicated in promoting cell survival, migration, and invasion (Zeng et al. 2002) (Fig. 8.2).

## 8.6 Tie/Ang

The Angiopoietin/Tie system acts as a vascular-specific ligand/receptor system and plays an essential role in tumor-associated angiogenesis. The angiopoietin family includes four ligands, namely, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), angiopoietin-3 (Ang-3), and angiopoietin-4 (Ang-4), and two corresponding RTKs (Tie1 and Tie2). Ang-1 acts as an agonist, whereas Ang-2 acts as an antagonist, of the Tie2 receptor (Maisonpierre et al. 1997). Tie2 activation promotes vessel assembly and maturation by mediating EC survival signals and regulating the recruitment of mural cells. The Tie/Ang-1 system may function during vessel maturation and stabilization and Ang-1 is a prominent regulator of vascular development (Lee et al. 2009; Machein et al. 2004). In contrast, Ang-2 is produced by ECs and acts as an autocrine antagonist of Ang-1-mediated Tie2 activation. Ang-2 is an angiogenic factor that antagonizes Ang-1 activity by competitively inhibiting the binding of Ang-1 to its cognate endothelial receptor, Tie2, causing vasculature destabilization. Ang-2 also acts in concert with VEGF to regulate vessel growth (Hu et al. 2006) (Fig. 8.3).





**Fig. 8.3** The major Ang/Tie signaling pathways in glioma angiogenesis and migration. Angiopoietin (Ang), growth factor receptor-bound protein 2 (GRB2), phosphatidylinositol 3-kinase (PI3K), rat sarcoma oncogene homolog (RAS), SH2 domain-containing phosphatase (SHP2), focal adhesion kinase (FAK), endothelial nitric oxide synthase (eNOS), docking protein R (Dok-R), extracellular receptor kinase (ERK), p21-activated kinase (PAK)

The role of Ang-1 in tumor-associated angiogenesis remains controversial because some reports imply that Ang-1 induction impairs angiogenesis and inhibits tumor growth whereas other authors suggest that Ang-1 overexpression promotes tumor growth in some animal models (Hawighorst et al. 2002; Hayes et al. 2000; Machein et al. 2004). However, Machein et al. reported that Ang-1 can promote tumor angiogenesis in a rat glioma model (Machein et al. 2004).

Ang-2 immunoreactivity is higher in malignant gliomas than in low-grade gliomas, which documents a strong correlation between the expression of Ang-2 and increasing glioma tumor grade (Koga et al. 2001). Increasing data suggest that Ang-2 expression is negatively correlated with vessel maturation in malignant gliomas and that VEGF expression is positively correlated with vessel maturation in low-grade gliomas.

Conflicting results have been reported in the literature regarding the role of the Tie2/Ang system in tumor angiogenesis. The tyrosine kinase receptor Tie2 was initially reported as a specific vascular receptor present in both normal and tumoral ECs, including ECs in astrocytomas, and its levels correlate positively with increasing malignancy (Liu et al. 2010; Stratmann et al. 1998). Tie2 is also expressed in glioma cells and brain tumor stem cells present in malignant gliomas and its expression and activation increases with increasing astrocytoma malignancy grade (Zadeh et al. 2004b).

Studies on *in vivo* human glioma biopsies have showed that Ang-2, MMP-2, MT1-MMP, and laminin 5 $\gamma$ 2 are co-overexpressed in the invasive areas but not in the central regions of the glioma tissues. *In vitro* data also demonstrated that Ang-2 promoted the expression and activation of MMP-2, MT1-MMP, and laminin 5 $\gamma$ 2, which may be essential for malignant glioma invasiveness (Brinckerhoff and Matrisian 2002; Lohi 2001). In addition, inhibition of Tie2 activation significantly decreased GBM xenograft growth by disrupting tumor vascularity (Zadeh et al. 2004b).

Accumulating evidence suggests that Tie2 activation regulates angiogenesis in a highly context- and tissue-dependent manner and closely collaborates with VEGF and possibly with other angiogenesis regulators (Gale et al. 2002; Zadeh et al. 2004b). Activation of the Tie/Ang functional axis promotes glioma proliferation and migration through various downstream effectors, including FAK, VEGF-A, PI3K,  $\alpha_v\beta_1$ , and extracellular signal-regulated kinase 1/2 (ERK1/2) (Brinckerhoff and Matrisian 2002; Lee et al. 2009; Zadeh et al. 2004b). Following activation of the Tie/Ang pathway, the FAK signaling pathway plays an essential role in malignant cell migration. The activation of Tie-2 induces VEGF-A to bind its EC-specific receptors, VEGFR1 and VEGFR2, which induces internalization of vascular endothelial cadherin (Zadeh et al. 2004a).

Furthermore, Tie receptor-independent signaling and non-vascular Ang effects can produce other intracellular signaling outcomes. ECs can adhere to immobilized Ang via the  $\alpha_v\beta_1$  integrin and FAK signaling pathway to induce glioma cell invasion by stimulating MMP-2 expression (Hu et al. 2006). The activation of the Tie-2/Ang-4 functional axis promotes the *in vivo* growth of human GBM cells by promoting tumor angiogenesis and directly activating ERK1/2 in GBM cells (Brinckerhoff and Matrisian 2002).

Although the preceding studies focused on elucidating the role of Ang-2 in cancer biology, its exact role in glioma angiogenesis remains elusive. A recent report demonstrated that Ang-2 significantly enhances vascular growth and induces aberrant pathological changes in malignant astrocytomas; this report also found that Ang-2 is not consistently elevated throughout all growth stages of malignant astrocytomas (Zadeh et al. 2010).

Although Ang-1 and Ang-2 play known roles in tumor angiogenesis, it is unknown how Ang-4 affects GBM angiogenesis progression and the mechanism underlying its effects. Brunckhorst et al. found a novel mechanism of Ang-4 on glioma progression that directly activates the ERK1/2 kinase pathway (Brunckhorst et al. 2010). Taken together, these data indicate that the Tie-2/Ang-4 functional axis can be considered an attractive therapeutic target for GBM.

## 8.7 Axl/Gas6

The receptor tyrosine kinase Axl, a member of the Tyro3, Axl, and Mer family of receptor tyrosine kinases (TAMRs), is characterized by an extracellular domain consisting of two immunoglobulin-like domains in juxtaposition to two fibronectin type III domains (Janssen et al. 1991). TAMRs, in particular Axl, have transforming properties: overexpression of a truncated version of Axl in premalignant cells is sufficient to induce tumors in mice (Zhang et al. 1996). Growth arrest-specific gene 6 (Gas6), which is the natural ligand of Axl, was discovered because its expression is upregulated in fibroblasts under growth-arrest conditions (Manfioletti et al. 1993). Axl/Gas6 signaling has been shown to regulate survival, proliferation, and migration in a variety of cells *in vitro* including tumor-derived cell lines of epithelial, mesenchymal, and hematopoietic origin (Hafizi and Dahlback 2006).

Axl and Gas6 are overexpressed in human gliomas of malignancy grades WHO II to IV. In contrast, Axl staining was not detectable in non-neoplastic brain tissue and Gas6 was strongly expressed in neurons (Hutterer et al. 2008). The receptor tyrosine kinase Axl is a mediator of glioma growth and invasion. Axl is predominantly expressed in pseudopalisading glioma cells, which are characterized by an accumulation of tumor cells around necrotic areas. Furthermore, an accumulation of Axl-positive tumor cells was observed adjacent to microvascular neoformations, which is a characteristic feature of invading glioma tumor cells spreading along perivascular regions. Inhibition of Axl signaling by overexpression of a dominant-negative receptor mutant suppressed experimental gliomagenesis and resulted in long-term survival of mice after intracerebral glioma cell implantation when compared with Axl wild-type transfected tumor cells. Inhibition of Axl signaling interfered with cell proliferation (30 % inhibition versus Axl wild-type), glioma cell migration (90 % inhibition versus Axl wild-type), and invasion (79 % inhibition versus Axl wild-type) However, an analysis of tumor vessel density and

diameter failed to reveal an attenuated tumor vasculature as an explanation for the reduced tumorigenesis in Axl dominant-negative cells (Vajkoczy et al. 2006). GBM patients with high Axl expression and Axl/Gas6 coexpression showed a significantly shorter time to tumor progression and poorer overall survival, indicating that Axl and Gas6 expression predict poor prognosis in GBM patients (Hutterer et al. 2008).

## 8.8 DDR1/Collagen

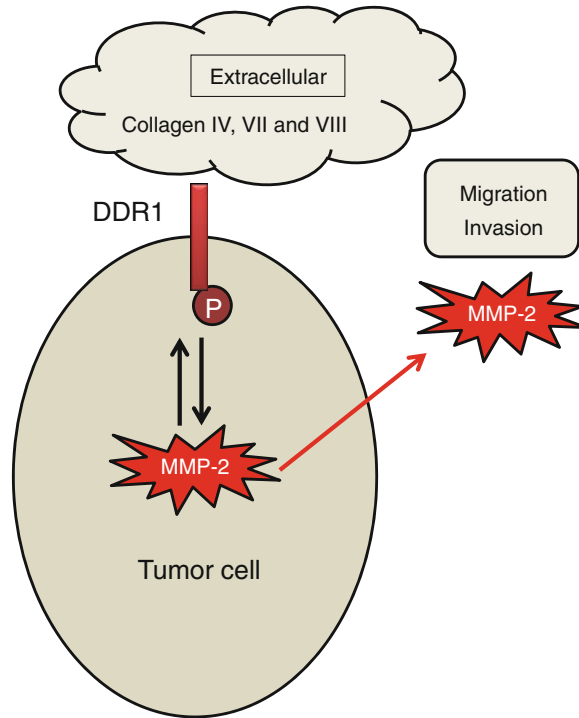
Discoidin domain receptor 1 (DDR1) tyrosine kinases constitute a family of non-integrin collagen receptors that contain a discoidin homology region in the ectodomain (Alves et al. 1995). DDR1, which is mainly expressed in epithelial cells, is primarily activated by collagens I to IV and VIII and facilitates cell adhesion (Vogel et al. 1997). DDR1 has five isoforms generated by alternative splicing: DDR1a, b, c, d and e. DDRs have been implicated in the expression of pro-inflammatory mediators and matrix-degrading enzymes and play an important role in migration, proliferation, ECM remodeling, and wound repair (Vogel et al. 2006).

In GBM, collagen IV is present in virtually all tumor vessels, in some giant glioma cells, and in tumor cells around vascular proliferations. Collagens VII and VIII are absent from normal brain but may be expressed in glioma tissues supporting DDR1 signaling (Senner et al. 2008). Furthermore, DDR1 is overexpressed in glioma. Although overexpression of either DDR1a or DDR1b in cell-based glioma models caused increased cell attachment, glioma cells overexpressing DDR1a exhibit enhanced invasion and migration concomitant with increased levels of MMP-2. Inhibition of MMP activity suppressed DDR1a-stimulated cell-invasion and inhibition of DDR1 reduced DDR1a-mediated invasion and enhanced adhesion of DDR1a and DDR1b overexpressing cells. DDR1a plays a critical role in inducing tumor cell adhesion and invasion, and this invasive phenotype is caused by activation of MMP-2 (Ram et al. 2006; Yamanaka et al. 2006) (Fig. 8.4). DDR1 expression is more closely correlated with survival than histological grade in gliomas, suggesting that DDR1 expression might be a better predictive factor of patient survival than WHO grading (Yamanaka et al. 2006).

## 8.9 Eph/Ephrin

Erythropoietin-producing human hepatocellular carcinoma (Eph) receptors comprise the largest family of RTKs in mammals. Eph receptors have been divided into an EphA subclass (nine members) and an EphB subclass (six members) on the basis of extracellular domain sequence homology and ligand affinity (Gale et al. 1996). Ephrin ligands are also transmembrane proteins and have been divided into two subclasses: glycosylphosphatidyl-inositol (GPI)-linked ephrin-As (five members), which are anchored to the cell membrane, and transmembrane ephrin-Bs (three members). Ephrin-As preferentially bind to EphA receptors, while ephrin-Bs preferentially bind to EphB receptors, although promiscuity has been observed.

Eph/ephrins form an essential cell-cell communication system capable of bi-directional intracellular signaling between adjacent cells, where receptor signaling is designated “forward” and ephrin signaling is “reverse” (Heroult et al. 2006; Kullander and Klein 2002). Eph/ephrin members are plentiful and their relationships are complex. Generally, Eph/ephrin interactions are repulsive because cells containing a given Eph are repelled by cells containing the corresponding Ephrin. Through this mechanism, the Eph/ephrin system plays a role in numerous biological processes including cell adhesion and migration during development, especially in the central nervous system (Wimmer-Kleikamp and Lackmann 2005). Recently, a role for the Eph/ephrin system has also emerged in cancer, especially in the area of invasive behavior (Campbell and Robbins 2008).

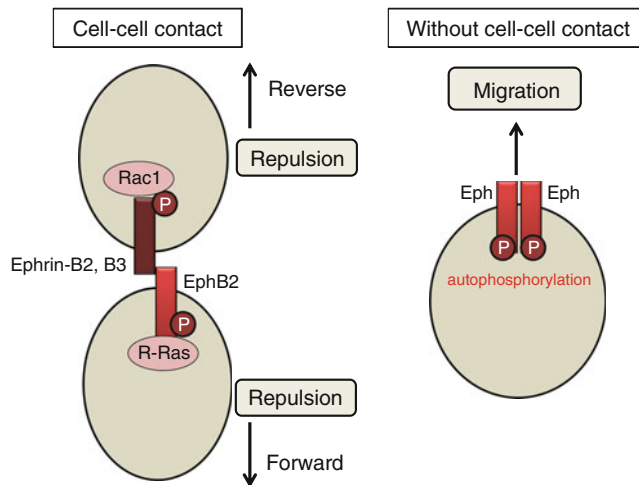


**Fig. 8.4** DDR1 signaling in glioma. In GBM, DDR1 is overexpressed and activated by collagen IV which is present in virtually all tumor vessels, as well as in some giant glioma cells and in tumor cells around vascular proliferations; also collagens VII and VIII may be expressed in glioma tissues supporting DDR1 signaling. Glioma cells overexpressing DDR1a display enhanced migration and invasion associated with the increase levels of matrix metalloproteinase 2 (MMP-2). (DDR1, discoidin domain receptor 1)

Genes for Eph/ephrins are overexpressed or differentially expressed in numerous human cancers (Surawska et al. 2004). Mounting evidence documents a strong correlation between the expression and phosphorylation levels of many Eph/ephrin family members and increasing glioma tumor grade, suggesting that elevated Eph/ephrin expression levels may be diagnostic for GBM and reduced patient survival rates (Nakada et al. 2011). Based on the microarray data obtained from two distinct GBM cell phenotypes (invading cells and tumor core cells) collected from GBM specimens, pathway enrichment analysis indicated that EphB/ephrin-B is the most tightly linked system to the invading cell phenotype (Nakada et al. 2010). According to these results, it is likely that the Eph/ephrin system contributes to glioma invasion. In spite of the overexpression of Eph/ephrin members in glioma, no evidence of gene amplification or mutation has been reported.

Numerous Ephs and ephrins have been noted to influence or correlate with the malignant behavior of cancer cells. In glioma, EphA/ephrin-A was associated with proliferation (Wykosky et al. 2005) whereas EphB/ephrin-B was involved in invasion and neovascularization (Erber et al. 2006). EphB2, ephrin-B2, and ephrin-B3 mRNA levels were shown to significantly increase with histological grade in glioma and contribute invasive properties in GBM (Nakada et al. 2004, 2006, 2010). Additionally, a high ephrin-B2 level was shown to confer poor survival (Nakada et al. 2010). The co-expression of EphB2 and ephrin-B in GBM cells suggests the existence of an EphB/ephrin-B interaction through cell–cell contact in GBM. Data showing that glioma invasion is inhibited by blocking Eph/ephrin suggest that the Eph/ephrin system is a potential therapeutic target for invasive glioma.

Different Eph/ephrin molecules are conceivably linked to different intracellular signaling pathways in a cell-type-specific manner, which allows this system to perform a variety of functions. The key



**Fig. 8.5** Putative model of Eph/ephrin function in glioma. Signaling induced by cell-cell contact via EphB/ephrin-B induces repulsion. Ligand independent signaling via autophosphorylation of Eph without ephrin stimulation can also promote the invasion without cell-cell communication

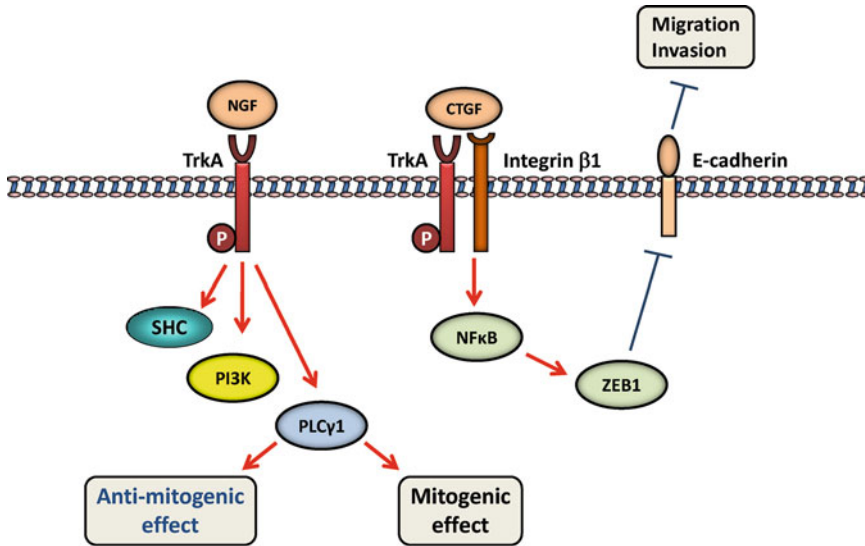
signaling molecules in the invasion-signaling pathway induced by EphB/ephrin B in GBM appear to be small GTPases such as Rac and Ras. Ephrin-B2 and ephrin-B3 can activate EphB2 through cell-cell contact, inducing invasion via EphB2 forward signaling. A previous study showed that EphB2 plays a functional role in promoting GBM cell invasion by eliciting signaling through R-Ras and affecting integrin activity (Nakada et al. 2005). In contrast, reverse signaling of the ephrin-B2 and ephrin-B3 ligands was demonstrated to be an important factor in the regulation of glioma cell invasion through the Rac1 GTPase (Fig. 8.5).

A greater depth of investigation has occurred with respect to involvement of the Eph/ephrin subsystem in invasion. Fundamental effect of Eph/ephrin necessitates direct cell-cell contact. However, it was recently revealed that ligand-independent signaling via autophosphorylation of Eph without ephrin stimulation can promote invasion without cell-cell communication (Miao et al. 2009) (Fig. 8.5). EphB2 was overexpressed in invading GBM cells compared with stationary cells in the tumor core, suggesting that EphB2 is autophosphorylated in invading glioma cells (Nakada et al. 2004, 2006). This indicates that invading glioma cells overexpressing Eph/ephrin far from the tumor can migrate by themselves without interaction of other cells.

## 8.10 TrkA

Neurotrophic tyrosine kinase receptor type 1 (TrkA) is the high affinity receptor for nerve growth factor (NGF), neurotrophin-3, and neurotrophin-4/5. Phosphorylated TrkA may play an important role in mitotic spindle assembly because it is colocalized with  $\alpha$ -tubulin at the mitotic spindle from prophase to anaphase, whereas in interphase, phosphorylated TrkA is localized on the membrane and processes of glioma cells (Zhang et al. 2005).

TrkA is strongly expressed in the subpopulation of highly infiltrating glioma cells *in vivo* but not in the glioma cells that remain within the bulk of the tumor. Thus, TrkA expression is dependent on both the cell type and the location within the tumor (Edwards et al. 2011). TrkA activation typically leads to the activation of survival- and growth-mediating pathways through the cytoplasmic proteins SHC, PI3K, and PLC- $\gamma$ 1 (Escalante et al. 2000; Meakin et al. 1999). GBM cell growth can be enhanced



**Fig. 8.6** TrkA signaling pathways in glioma. GBM cell growth can be enhanced by TrkA receptor (neurotrophic tyrosine kinase receptor type 1) phosphorylation activated by NGF (nerve growth factor). TrkA activation leads to the activation of survival and growth mediating pathways through cytoplasmic proteins SHC, PI3K and PLC- $\gamma$ 1. Switching between anti-mitogenic and mitogenic TrkA signaling is controlled by PLC- $\gamma$ 1 activity. Glioma initiating/stem cells require integrin  $\beta$ 1–TrkA complex for connective tissue growth factor (CTGF) signaling; binding of the complex with CTGF causes NF- $\kappa$ B-mediated activation of the ZEB-1 promoter with subsequent induction of the ZEB-1 transcriptional repressor resulting in decreased expression of E-cadherin, accordingly enhancing glioma cell invasion and migration

by NGF acting via TrkA receptor phosphorylation (Singer et al. 1999). Switching between anti-mitogenic and mitogenic TrkA signaling is controlled by PLC- $\gamma$ 1 activity (Ye et al. 2000, 2002) (Fig. 8.6). Consequently, it was shown that TrkA expression in GBMs attenuates tumor progression *in vivo* (Rabin et al. 1998) by inducing differentiation of tumor cells from undifferentiated glioma to oligodendrocytes (Pflug et al. 2001).

Glioma initiating/stem cells require the cell surface protein receptors integrin  $\beta$ 1 and TrkA, which constitute the integrin  $\beta$ 1–TrkA complex, for connective tissue growth factor (CTGF) signaling. The integrin  $\beta$ 1–TrkA complex can bind to CTGF and cause nuclear factor-kappa B (NF- $\kappa$ B)-mediated activation of the ZEB-1 promoter with subsequent induction of the ZEB-1 transcriptional repressor, resulting in decreased expression of E-cadherin and subsequent enhancement of glioma cell invasion and migration (Fig. 8.6). TrkA knockdown resulted in decreased CTGF-induced cell migration and the absence of tumor cell invasion into normal mice cortex. However, TrkA knockdown did not affect CTGF-induced proliferation or the clonogenicity of the glioma stem cells *in vitro*. Thus, CTGF, TrkA, and NF- $\kappa$ B may be potential therapeutic targets to alleviate tumor cell infiltration (Edwards et al. 2011).

## 8.11 Cross-Talk

As described above, many RTKs lead to the signaling of PI3K/Akt and Rac1 in invading glioma cells, suggesting that these molecules confer critical downstream signaling for invasion. A previous study showed coexpression of multiple activated RTKs in individual dissociated cells from a primary GBM (Stommel et al. 2007). Accordingly, multiple RTKs may be simultaneously or sequentially used by GBM cells to maintain invasion-signaling pathways via molecules such as PI3K/Akt and Rac1.

Given that individual cells express multiple RTKs, it is reasonable to speculate that these RTKs are interacting with each other. For example, the c-Met receptor is strongly phosphorylated as a function of EGFRvIII receptor levels, suggesting the presence of cross-talk between c-Met and EGFRvIII signaling, although the intermediary molecule has not yet been elucidated (Huang et al. 2007). The Axl RTK follows a similar phosphorylation response as a function of EGFRvIII levels (Huang et al. 2007). It was previously reported that EGFR and EphA2 are expressed in GBM 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 (Ramnarain et al. 2006). Previous studies reported 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 (Fukai et al. 2008).

In such a multiple-input system with cross-talk among RTKs, a single-agent of anti-RTK inhibition might be incapable of sufficiently suppressing invasion signaling, resulting in insensitivity to invasion inhibition by any single agent and a lack of clinical efficacy. It is anticipated that combinations of drugs against different activated RTKs or single drugs with inhibitory activities against multiple activated RTKs will have more favorable outcomes.

## 8.12 Targeting Receptor Type Tyrosine Kinases

As mentioned in the previous paragraphs, TKIs that target multiple signaling pathways and critical growth factors essential for tumor progression have become a major focus of interest in various clinical studies. Although all studies remain experimental, several of these specific molecular agents that are most expectant or have been widely evaluated are reviewed below (Table 8.2).

### 8.12.1 Targeting EGFR

#### 8.12.1.1 Gefitinib

Gefitinib is a low molecular weight, selective inhibitor of EGFR tyrosine kinase and was the first drug of this type. Although an early study suggested that gefitinib was active in patients with malignant gliomas (Franceschi et al. 2007; Mellinghoff et al. 2005), gefitinib therapy showed limited efficacy in multicenter phase II studies in patients with malignant glioma (Franceschi et al. 2007; Hegi et al. 2011; Rich et al. 2004), either for recurrent disease or as part of the initial treatment regimen. Because EGFR kinase domain mutations, which are associated with the greatest sensitivity to EGFR inhibitors (Pedersen et al. 2005), are uncommon in malignant gliomas (Lassman et al. 2005), it remains to be determined whether other molecular alterations involving EGFR signaling pathways occur selectively in treatment responders.

#### 8.12.1.2 Erlotinib

Erlotinib is an orally active, reversible EGFR TKI. Because erlotinib is metabolized by the cytochrome P450 isoenzymes 3A4 (70 %) and CYP 1A2 (30 %), patients taking enzyme-inducing anti-epileptic drugs are not eligible for this treatment (Raizer et al. 2010). Similar to gefitinib, clinical studies examining the therapeutic efficacy of erlotinib have so far failed to demonstrate a major therapeutic break-through

**Table 8.2** Summary of phase II studies in various tyrosine kinase inhibitors and related agents

Primary target	RTKI	Other target	Objects	Median OS (week)	OS (%)	Median PFS (week)	PFS (%)	References
EGFR	Gefitinib containing regimen		Newly diagnosed GB after RTx		54.2 (12 m)		16.2 (12 m)	(Uhm et al. 2011)
			Newly diagnosed pediatric brainstem glioma after RTx		56.4 (12 m)	32.1	20.9 (12 m)	(Pollack et al. 2011)
			Recurrent GB after RTx	24.6–25.1	50.0 (6 m)	8.4–17.0	13.0–14.3 (6 m)	(Franceschi et al. 2007; Hegi et al. 2011; Rich et al. 2004)
	Erlotinib containing regimen		Newly diagnosed GB after RTx	37.3	–	12.2	30.0 (6 m)	(Peereboom et al. 2010)
			Recurrent GB after RTx	26.0	11.4 (6 m)	8.7–31.2	3.0–11.4 (6 m)	(Brown et al. 2008; Lassman et al. 2005; Mellingerhoff et al. 2005; Raizer et al. 2010; Van Den Bent et al. 2009)
	(125) I-Mab425		Recurrent AG after RTx	30.4	–	8.7	27.0 (6 m)	(Raizer et al. 2010)
			GB after RTx	63.1	62.5 (12 m), 25.5 (24 m)	–	–	(Li et al. 2010)
	Nimotuzumab		Recurrent pediatric brainstem glioma	82.0	–	–	–	(Massimino et al. 2011; Saurez et al. 2009)
			Recurrent malignant glioma after RTx	82.5	82.0 (6 m), 64.0 (12 m)	–	64.0 (6 m)	(Saurez et al. 2009)





in the setting of GBM (Haas-Kogan et al. 2005; Mellingshoff et al. 2005; Raizer et al. 2010; Van Den Bent et al. 2009), including newly diagnosed GBM treated with temozolomide (TMZ) and radiotherapy (Peereboom et al. 2010). Although Raizer and colleagues reported that the development of a rash during the initial erlotinib administration cycle correlates with survival in patients with non-progressive GBM after radiotherapy, the significance of this finding remains unclear (Raizer et al. 2010).

#### 8.12.1.3 <sup>125</sup>I-mAb 425

<sup>125</sup>I-mAb 425, an <sup>125</sup>I-labeled anti-EGFR 425 murine monoclonal antibody (mAb), is an IgG2a isotype developed from mice immunized with A-431 epidermoid carcinoma cells. mAb 425 binds to the tumor and produces anticarcinogenic effects mediated by direct cell growth inhibition, complement-dependent cytotoxicity, and activation of the humoral response. Initial clinical studies have shown survival benefits of adjuvant <sup>125</sup>I-425 mAb in GBM patients (Emrich et al. 2002; Quang and Brady 2004). In a phase II study of 192 patients with GBM treated with anti-EGFR <sup>125</sup>I-mAb 425 radioimmunotherapy, the reported survival was 15.7 months and treatment was safe and well tolerated (Li et al. 2010).

#### 8.12.1.4 Nimotuzumab

Nimotuzumab (h-R3), a humanized monoclonal antibody directed against the EGFR, consequently inhibits tyrosine kinase activation. Recently, several reports of pediatric diffuse intrinsic pontine glioma treated with nimotuzumab showed a relatively favorable prognosis (Lam et al. 2009; Mateos et al. 2011; Saurez et al. 2009). Thus, nimotuzumab is currently being evaluated in conjunction with radiotherapy in a phase III trial in children with diffuse pontine glioma and adult GBM.

#### 8.12.1.5 Cetuximab

Cetuximab, IMC-C225, is a chimeric (mouse/human) monoclonal IgG1 antibody that binds to EGFR with high specificity and affinity. Encouragingly, cetuximab enhanced the cytotoxicity produced by radiation therapy *in vivo* in EGFR-amplified GBM (Belda-Iniesta et al. 2006; Eller et al. 2005). In 2006, however, a phase II trial of cetuximab in 55 recurrent high-grade glioma patients revealed no significant correlation between response, survival, and EGFR amplification (Neyns et al. 2009).

#### 8.12.1.6 Lapatinib

Lapatinib is a dual TKI that interrupts the HER2/neu (ErbB2) growth receptor pathway. It is used in therapy for HER2-positive breast cancer. However, a phase I/II trial of lapatinib in patients with relapsed GBM failed to show significant activity of this agent independent of the presence of EGFRvIII mutation and PTEN immunohistochemical status (Thiessen et al. 2010).

### 8.12.2 Targeting PDGFR

#### 8.12.2.1 Imatinib

Imatinib mesylate (Gleevec; formerly known as STI571) is a potent inhibitor of the PDGFR $\alpha$ , PDGFR $\beta$ , Bcr-Abl, c-Fms, and c-Kit tyrosine kinases. Its antitumor activities in chronic myelogenous leukemia and gastrointestinal stromal tumors result from the inhibitions of Bcr-Abl (Druker et al.

2001) and c-Kit (Demetri et al. 2002), respectively. An initial study showed that imatinib mesylate plus hydroxyurea was well tolerated and associated with durable antitumor activity in some patients with recurrent GBM (Reardon et al. 2005). However, more extensive phase II and III studies have shown only minimal evidence of single agent activity (Dresemann et al. 2010; Raymond et al. 2008; Wen et al. 2006).

### 8.12.2.2 Sunitinib

Sunitinib malate is an oral small-molecule inhibitor of VEGFRs, PDGFRs, c-Kit, Flt3, and RET kinases (Chow and Eckhardt 2007). Sunitinib has substantial clinical activity against hypoxia-inducible factor (HIF)/VEGF-dependent, PDGFR-dependent, and KIT-dependent cancers. Recently, a phase II study of sunitinib failed to demonstrate a relevant clinical benefit from single-agent sunitinib (37.5 mg/day) in patients with alkylator-refractory recurrent glioma. Furthermore, substantial treatment-related toxicity was observed in several patients (Neyns et al. 2011). Although sunitinib affected the glioma vasculature in a small subgroup of patients, no objective tumor responses were observed regarding apparent reduced cerebral blood-flow and blood-volume within the lesion compared with the normal brain or reduced gadolinium enhancement of the tumor. A possible explanation for the failure of sunitinib to have meaningful clinical activity is the lower potency of sunitinib to selectively inhibit VEGF/VEGFR signaling within the tumor vasculature compared to the VEGF-targeted mAb bevacizumab (Friedman et al. 2009) or the more potent VEGFR-specific small-molecule TKI cediranib (Batchelor et al. 2010a, b).

### 8.12.2.3 Dasatinib

Dasatinib is an aminotriazole analog with high specificity for several kinases including Bcr-Abl, Src, c-Kit, PDGFR $\beta$ , and EphA2, which was approved for use in patients with chronic myelogenous leukemia after imatinib treatment and Philadelphia chromosome-positive acute lymphoblastic leukemia. Dasatinib appears to be a more potent inhibitor of Bcr-Abl than imatinib (Tokarski et al. 2006). Recently, investigators have shown that Src is frequently phosphorylated in GBM cell lines such as T98G and U87 compared with normal tissue and is activated in human GBM tumors (Du et al. 2009). These findings support the possible role of dasatinib for malignant gliomas, and future clinical trials will further assess the clinical value of SRC inhibition with dasatinib incorporating TMZ and other cytotoxic agents.

## 8.12.3 *Multi-Kinase Inhibitors*

### 8.12.3.1 Cediranib

Cediranib (AZD2171) is a potent oral inhibitor of all VEGF receptor tyrosine kinases and PDGF receptors (Batchelor et al. 2007). Cediranib caused a structural and functional normalization of tumor vasculature in all 16 patients with recurrent GBM and resulted in a significant reduction of tumor-associated vasogenic edema as measured by MRI techniques. This effect was paralleled by a potent steroid-sparing effect in most patients (Batchelor et al. 2007). In a subsequent phase II study, partial responses were seen in 57 % of patients based upon tumor measurements (Batchelor et al. 2010b), and the median progression-free survival (PFS) and the median overall survival (OS) were 32.4 weeks and 16.7 weeks, respectively (Table 8.2), with manageable toxicity. Based upon these results, a phase III study was conducted in 325 patients with recurrent GBM (Batchelor 2010a). Although cediranib

treatment regimens resulted in a statistically significant decrease in steroid use and a reduced contrast enhancing area on neuroimaging, there was no statistically significant improvement in PFS, the primary endpoint of the trial.

### 8.12.3.2 Sorafenib

Sorafenib is a multi-target oral TKI with inhibitory effects on the VEGF receptor, PDGF receptor, and the Ras/Raf signaling pathway (Wilhelm et al. 2004). Despite potentially complementary direct and indirect mechanisms of anti-tumor activity, Reardon et al. demonstrated that sorafenib combined with daily TMZ has minimal activity as a salvage regimen for recurrent GBM patients in their single-arm phase II study (Reardon et al. 2011). Meanwhile, Hainsworth et al. also failed to demonstrate the efficacy of treatment when compared with the results expected with standard therapy in newly diagnosed GBM (Hainsworth et al. 2010). A plausible explanation for this low effectiveness is the low to moderate ability of sorafenib to penetrate into the brain. However, the blood brain barrier (BBB) is compromised in patients with GBM and it is unlikely that sorafenib would have lower penetration than bevacizumab, a large protein. In general, the addition of small molecule TKIs to chemotherapy has not consistently improved treatment results, even when both components of therapy have individual efficacy.

### 8.12.3.3 Vandetanib

Vandetanib (ZD6474) is a potent oral TKI for various RTKs, in particular VEGFR2 and EGFR. A phase I study of 35 children with newly diagnosed diffuse intrinsic pontine glioma treated with vandetanib reported 1- and 2-year OS outcomes of 37.5 %  $\pm$  10.5 % and 21.4 %  $\pm$  11 %, respectively. Three patients remained alive with PFS for more than 2 years. The recommended phase II dose of vandetanib in children is 145 mg/m<sup>2</sup> per day (Broniscer et al. 2010). Several phase II studies, including those in recurrent and newly diagnosed malignant glioma, are currently underway.

### 8.12.3.4 Cabozantinib (XL-184)

Recently, interim results have been reported from a phase II study of Cabozantinib (XL184) treatment in previously treated progressive GBM. Cabozantinib is an oral inhibitor of multiple RTKs that includes VEGFR2 as the main target followed by c-Met, RET, c-kit, Flt3, Tie-2, and Axl (Wen 2010; Yakes et al. 2011). In this study, the median PFS in antiangiogenic-naive cohorts was 16 weeks (Table 8.2). In total, 61 % of patients on corticosteroids at baseline had a reduction in corticosteroid dose of at least 50 %. The investigators concluded that XL184 demonstrates encouraging clinical activity in patients with progressive GBM. Cabozantinib, currently in phase III clinical trials, is a promising agent for inhibiting tumor angiogenesis and metastasis in glioma, especially in cases of dysregulated Met and VEGFR signaling.

## 8.13 Prospective

Future studies are required to more precisely establish the molecular mechanisms and specific downstream signaling elements that contribute to the cooperative or synergistic interactions of RTK signaling pathways in invading glioma cells. Further studies on the manipulation of the RTK systems involved in invasion will aid in engineering GBM therapies and in elucidating the complexity and additional

functional implications of RTK systems. Moreover, it is of great therapeutic interest to define invasion-associated RTKs that could be targeted for blocking invasion. These works should help to develop novel potential pharmacological agents to modulate invasion processes and thereby counteract the activation of invasion-signaling pathways and promotion of invasion. The data obtained from GBM patients treated with TKIs should confirm the therapeutic benefit of TKIs and the safety of selectively targeting RTKs, alone or in combination with the current conventional therapies. The effort to combat GBM, along with emerging data regarding the underlying molecular invasion circuitry and the development of specific TKIs, will result in the development of an array of new treatment approaches.

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