

Targeting the epithelial to mesenchymal transition in glioblastoma: the emerging role of MET signaling

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Abstract: Glioblastoma multiforme (GBM) is the most common human primary brain malignancy and has a dismal prognosis. Aggressive treatments using maximal surgical resection, radiotherapy, and temozolomide result in median survival of only 14.6 months in patients with GBM. Numerous clinical approaches using small molecule inhibitors have shown disappointing results because of the genetic heterogeneity of GBM. The epithelial to mesenchymal transition (EMT) is a crucial biological process occurring in the early development stages of many species. However, cancer cells often obtain the ability to invade and metastasize through the EMT, which triggers the scattering of cells. The hepatocyte growth factor (HGF)/MET signaling pathway is indicative of the EMT during both embryogenesis and the invasive growth of tumors, because HGF potently induces mesenchymal transition in epithelial-driven cells. Activation of MET signaling or co-overexpression of *HGF* and *MET* frequently represents aggressive growth and poor prognosis of various cancers, including GBM. Thus, efforts to treat cancers by inhibiting MET signaling using neutralizing antibodies or small molecule inhibitors have progressed during the last decade. In this review, we discuss HGF/MET signaling in the development of diseases, including cancers, as well as updates on MET inhibition therapy.

Keywords: glioblastoma multiforme, epithelial to mesenchymal transition, MET signaling

Introduction

Glioblastoma multiforme (GBM) is the most common and aggressive form of adult brain tumor.¹ Although its incidence is relatively low, it has attracted attention because of its dismal prognosis. Despite the aggressive treatment regimen, which includes surgery and radiotherapy plus temozolomide, median survival is only 12–15 months in patients with GBM.^{2,3}

Improved understanding of the molecular pathogenesis of GBM has encouraged the adaptation of targeted molecular therapies to treat GBM.⁴ However, most clinical trials evaluating new drugs targeting cancer-specific signaling pathways have shown only minimal effects on the treatment of patients with GBM.⁴ A randomized controlled clinical trial assessing the antiangiogenic drug bevacizumab demonstrated that targeting angiogenesis increases tumor cell invasion in patients with GBM.⁵ Although molecular targeted agents potently inhibit receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, and vascular endothelial growth factor receptor, which are major survival and proliferation-promoting signaling molecules in GBM,^{4–7} treatment using a single agent has shown only minimal improvement, with response rates of 0%–15% of 6-month progression-free survival.⁸

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The epithelial to mesenchymal transition (EMT) is a biological process that allows immobile epithelial cells to undergo biochemical changes and induces a mesenchymal cell phenotype, including enhanced migratory properties, invasiveness, and resistance to apoptosis.^{9,10} The EMT is also associated with tumor cell invasion that leads to metastatic dissemination.¹¹ A study that investigated the association between mesenchymal markers and gliomas revealed a series of mesenchymal tissue-associated genes such as *YKL-40*, *TNC*, osteonectin, and *CD105* to be overexpressed in GBM biopsies.¹² A comprehensive gene expression analysis of 85 high-grade gliomas identified a subset of GBM tissues also overexpressing mesenchymal tissue-associated genes.¹³ *TWIST*, an EMT-associated molecule, also plays a key role in survival and invasion of human glioma cells.¹⁴

c-MET, a receptor tyrosine kinase, functions as a proto-oncogene by activating multiple cellular signal pathways that promote proliferation, migration, and invasion of cancer cells.¹⁵ Hepatocyte growth factor (HGF), a pleiotropic factor that promotes cell proliferation, survival, and motility, is a c-MET ligand.^{16,17} In addition, the MET tyrosine kinase induces glioma cell proliferation, survival, and migration.¹⁸ HGF/MET signaling also confers resistance to radiotherapy by promoting survival of glioma stem cells (GSCs).¹⁹ The *MET* oncogene is associated with the formation of neurospheres in mesenchymal and proneural subtypes of glioblastomas.²⁰ HGF/MET signaling is also associated with invasive growth phenotype, which is a characteristic of EMT in GBM.²¹

In this review, we discuss issues related to identification of the MET signaling pathway as a therapeutic target via inhibition of the EMT in GBM.

EMT in development and disease

The EMT was originally defined to be a biological process that transforms mesenchymal cells from epithelial cells in different embryonic tissues.²² Both *SNAIL* (*Snail*) and (*SNAIL2*) *Slug* are critical factors in the delamination process of neuronal tissue development.²³ Renal fibrosis is a characteristic kidney disease eventually leading to renal failure.²⁴ Accumulating evidence has demonstrated that the majority of interstitial fibroblasts are derived from the kidney epithelium. The EMT is also a major issue in patients undergoing peritoneal dialysis, because long-term dialysis enhances injury of the mesothelial lining, which leads to the EMT, including loss of E-cadherin and increased Snail expression.²⁵ In addition, the EMT is involved in anterior–subcapsular cataracts in humans.²⁶ Eye lens epithelial cells

undergo transdifferentiation into a myofibroblastic phenotype in combination with the production of type I and type III collagens, fibronectin, and tenascin.

EMT in human cancers

EMT in cancers

Epithelial cell plasticity is a hallmark of invasive and/or metastatic malignancies. Evidence indicates that EMT occurs at certain sites in primary tumors.²⁷ E-cadherin-negative cells from colon cancer are found at sites of tumor invasion and bud into the stroma, which contributes to local dissemination and metastasis of primary tumors. One study demonstrated that fibroblast-specific protein-1, together with a conversion signal for local formation of fibroblasts by the EMT, provokes acquisition of a metastatic phenotype in genetically engineered mice with breast cancer.²⁸ The EMT induced by ectopic *TWIST* expression promotes invasiveness, suppressing E-cadherin expression in hepatoma cell lines.²⁹ Irradiation-induced EMT confers invasive properties in endometrial cancer cells.³⁰

EMT in therapy resistance

The EMT also confers resistance to both radiotherapy and chemotherapy. Kajiyama et al³¹ discovered that paclitaxel-resistant cells, which develop following chronic exposure to paclitaxel, demonstrate cellular and molecular characteristics of the EMT. *Snail* and *Slug*, transcriptional repressors and inducers of the EMT, contribute to radio- and chemoresistance by inhibiting p53-mediated apoptosis in ovarian cancer cells.³² The endothelin-1/endothelin-A receptor axis is involved in chemoresistance by promoting the EMT in ovarian cancer cells in vitro and in vivo.³³ Stable expression of *SNAIL*, an EMT inducer, contributes to drug resistance and acquisition of stem-like characteristics in MCF10A cells.³⁴ Suda et al³⁵ determined that the EMT plays a part in the acquired resistance to EGFR tyrosine kinase inhibitors such as erlotinib in non-small-cell lung cancer (NSCLC) cells.³⁶

EMT in cancer stem cells

Reports have demonstrated that the EMT also helps maintain cancer stem cells. The EMT process promotes the emergence of cancer cells with mesenchymal traits essential for tumor invasion/metastasis and the self-renewal properties needed for colonization of a secondary tumor.³⁷ Stem cells derived from malignant breast tissue have a basal-like phenotype and highly upregulated expression of EMT-associated genes.^{38,39} Mani et al⁴⁰ found that EMT induction in immortalized human mammary epithelial cells promotes the acquisition

of stem cell-like properties, including formation of mammospheres and stem cell marker expression. Morel et al⁴¹ discovered that CD44⁺CD24^{-/low} mammary epithelial cells demonstrate an EMT phenotype, including loss of E-cadherin and gain of vimentin expression. They also demonstrated that treatment with transforming growth factor (TGF)- β , a potent EMT-inducing factor, induced the switch of CD24⁺ cells into stem-like CD24⁻ cells.

EMT molecular mechanisms

The EMT is characterized by the loss of polarity and down-regulation of epithelial cell-associated proteins, including E-cadherin, γ -catenin/plakoglobin, and Zo-1, along with induced expression of mesenchymal proteins, including smooth muscle actin, fibronectin, N-cadherin, and vimentin.^{42,43} Expression of these molecules enables epithelial cells to acquire a more flexible and migratory mesenchymal cell-like phenotype.²⁷

EMT-inducing signals in many cancers, notably HGF, EGF, platelet-derived growth factor, and TGF- β , are derived from the tumor-associated stroma (Figure 1). These ligands stimulate cells and activate a series of EMT-promoting transcription factors such as Snail, Slug, zinc finger E-box binding homeobox 1, and *TWIST*.^{9,27} A series of intracellular signaling networks, including extracellular regulated kinase, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), Akt, SMADs, β -catenin, and Ras pathways, is involved in the EMT process.⁴⁴ Cell surface molecules, including β 4 integrins and α 5 β 1 and α V β 6 integrins, are also associated with transmitting EMT-promoting signals.⁴⁵

TGF- β is an important inducer of the EMT in various cancers. SMAD proteins mediate signaling induced by TGF- β via the anaplastic lymphoid kinase (ALK)-5 receptor.^{46,47} p38 MAPK and RhoA also mediate TGF- β -induced EMT in mouse mammary epithelial cells.^{48,49} Active β -catenin, bound to the cytoplasmic tail of E-cadherin, accumulates

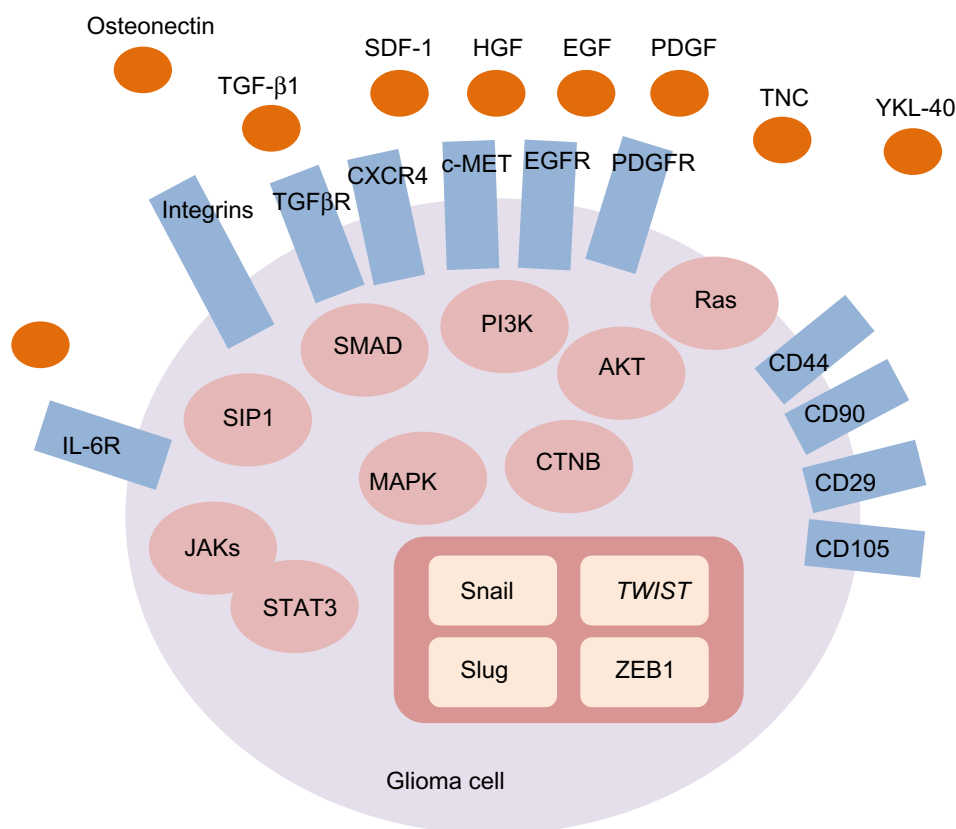


Figure 1 EMT-associated signaling molecules in glioma cell. Glioma-associated stromal molecules, including TNC, YKL-40, osteonectin, SDF-1, TGF- β 1, HGF, EGF, PDGF, and IL-6, trigger the EMT via stimulating corresponding receptors in glioma cells. Cell surface molecules, including IL-6R, TGF- β R, CXCR4, c-MET, EGFR, and PDGFR, initiate the oncogenic signaling cascade when they are activated by their specific ligands. β 4 integrins and α 5 β 1 and α V β 6 integrins are also associated with transmitting the EMT-promoting signals. In addition, glioma cells express the mesenchymal stem cell-associated surface markers such as CD29, CD44, CD90, and CD105. Intracellular signaling networks, including ERK, MAPK, PI3K, Akt, SMADs, β -catenin, and the Ras pathway, are involved in the EMT. These signaling networks activate a series of EMT-promoting transcription factors such as Snail, Slug, ZEB1, and *TWIST*.

Abbreviations: CXCR4, chemokine (C-X-C motif) receptor 4; EGF, epidermal growth factor; EMT, epithelial to mesenchymal transition; ERK, extracellular regulated kinase; HGF, hepatocyte growth factor; IL-6, interleukin-6; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; TGF- β 1, transforming growth factor β 1; TNC, tenascin C; ZEB1, zinc finger E-box binding homeobox 1.

in the nucleus, where it forms part of the Tcf/LEF complex. This β -catenin-mediated signaling pathway correlates with acquisition of the EMT and tumor invasiveness.⁵⁰ Loss of E-cadherin is induced by several EMT-promoting transcription factors such as Snail, Slug, SIP1, and E12.⁵¹ SIP1 in human epithelial cells induces morphological changes from the epithelial to mesenchymal phenotype.⁵² The transcription repressor SIP1, also known as ZEB2, suppresses E-cadherin expression by binding to the E-cadherin promoter along with Snail.⁵³ The major regulators of embryogenesis, *TWIST* 1 and *TWIST* 2, cooperate with Ras to transform embryonic fibroblasts. The basic helix–loop–helix regulatory factor *TWIST* 1 induces the EMT and metastatic dissemination of cancer cells by promoting Snail expression.^{54,55}

Xie et al⁵⁶ reported that interleukin (IL)-6 is capable of generating stem-like CD44⁺ cells by inducing the EMT in the T47D luminal breast cancer cell line. IL-6 also promotes EMT-related phenotypic changes and mesenchymal cell-specific gene expression by activating the Jak/Stat3/Snail signaling pathway in head and neck squamous cells and immortalized oral epithelial cells.⁵⁷ Recent evidence indicates that the IL-6/casein kinase 2 signaling pathway promotes EMT and cancer cell migration by stabilizing *TWIST* at the post-translational level.⁵⁸

EMT in malignant glioma

Overexpression of *TWIST*, an EMT-promoting factor, significantly enhances tumor cell invasion in a human glioma cell line (Table 1).¹⁴ One study demonstrated that primary glioblastoma and its stem cell lines express cellular and molecular characteristics of mesenchymal stem cells (MSCs).¹² Additionally, GBM cell lines largely express MSC-associated surface markers, including CD29, CD44,

CD90, and CD105. Genes associated with mesenchymal cells, such as *YKL-40*, *tenascin C (TNC)*, *osteonectin*, and *CD105*, were highly expressed in primary GBM tumors and glioma cell lines. Knockdown of signal transducer and activator of transcription 3 (STAT3), a master regulator of the EMT and cell migration, inhibits glioma cell infiltration and tumor growth in vivo.⁵⁹ EMT signature genes discovered in a study using mammary cancer tissue, including *TAGLN2*, insulin-like growth factor-binding protein 2 (*IGFBP2*), *IGFBP3*, periostin (*POSTN*), *TNC*, and *TGF- β 1*, are predictors for survival in patients with malignant glioma.⁶⁰ These genes are also upregulated in each of the GBM subtypes: mesenchymal, classical, neural, and proneural. Among the four GBM subtypes, the mesenchymal subtype demonstrates the highest correlation with the EMT-inducing genes.

Snail homology 1 (*SNAI-1*), an EMT-promoting factor, is upregulated in glioma specimens when compared with the normal brain tissue, and *SNAI-1* expression is higher in high-grade than in low-grade gliomas.⁶¹ Interference of *SNAI-1* inhibits the proliferation and migration of glioma cell lines, which confirms a critical role of the EMT in the migration and invasion of glioma cells.⁶² Another EMT inducer, SMAD-interacting protein-1, promotes invasion, migration, and clonogenicity of glioma cells.⁶³ The chemokine receptor CXCR4 has been regarded to mediate MSC-specific migration.⁶⁴ Silencing CXCR4 inhibits invasion of the U87 human glioma cell line by suppressing the EMT, and it also upregulates E-cadherin and decreases N-cadherin and vimentin expression.⁶⁵

MET signaling and EMT

c-MET is a receptor tyrosine kinase involved in a variety of cellular signaling pathways, including those associated with

Table 1 Importance of EMT-related molecules in malignant glioma

EMT-related molecules/genes	Functions	Importance in malignant glioma	Reference
TWIST	EMT-promoting factor	Enhanced invasion	14
CD29, CD44, CD90, CD105, YKL-40, TNC, and osteonectin	MSC-related proteins	Highly expressed in GBM cell lines	12
STAT3	A master regulator of EMT	Promoting infiltration and tumor growth	59
<i>TAGLN2</i> , <i>IGFBP2</i> , <i>IGFBP3</i> , <i>POSTN</i> , <i>TNC</i> , and <i>TGF-β1</i>	EMT signatures identified in breast cancers	Predictors for prognosis of GBM patients	60
SNAI-1	Promoting EMT	High expression in glioma. Expression level increased with grade. Promoting cell proliferation and infiltration	61,62
SIP1	An EMT inducer	Promoting invasion and clonogenicity	63
CXCR4	MSC-specific surface marker	Enhanced migration. Promoting EMT markers expression	65

Abbreviations: CXCR4, chemokine (C–X–C motif) receptor 4; EMT, epithelial to mesenchymal transition; GBM, glioblastoma multiforme; IGFBP, insulin-like growth factor-binding protein; MSC, mesenchymal stem cell; *POSTN*, periostin; SIP1, SMAD-interacting protein 1; *SNAI-1*, Snail homology 1; *TGF- β 1*, transforming growth factor- β 1; *TNC*, tenascin C.

proliferation, invasion, and self-renewal.¹⁵ This cell surface receptor is located in epithelial cells of various organs, including the liver, prostate, kidney, muscle, and bone marrow.⁶⁶ The extracellular portion of c-MET is composed of three domains: the Sema; plexins, semasphorins, and integrins; and the immunoglobulin–plexin–transcription domain.⁶⁷ The intracellular portion of the c-MET contains a tyrosine kinase catalytic domain with a flanked juxtamembrane and a carboxy-terminal sequence.¹⁵ This intracellular portion of the c-MET receptor consists of catalytic tyrosines Y1234 and Y1235, which positively regulate the enzymatic activity of c-MET, whereas the juxtamedullary portion contains Y1003, which negatively regulates c-MET signaling (Figure 2).

HGF, also known as scatter factor (SF), is the ligand for c-MET that is considered a motility factor and an SF.^{16,68} HGF acts as a pleiotropic factor that promotes proliferation,

motility, scattering, and differentiation. HGF binding to c-MET induces receptor homodimerization and phosphorylation of tyrosine residues (Y1234 and Y1235) within the catalytic loop, followed by phosphorylation of Y1349 and Y1356 in the carboxyl-terminal tail.^{69,70} When these tyrosine residues are phosphorylated, various adaptor and effector molecules are recruited, including growth factor receptor bound protein 2, Src homology-2-containing PI3K, phospholipase C γ , Src homology domain-containing 5' inositol phosphatase, and STAT3 (Figure 2).^{70–73} The c-MET receptor interacts with multiple signaling adaptors and cell surface receptors to mediate a variety of biological responses. Recent large-scale proteomic studies have demonstrated notable intricacies in the HGF/c-MET signaling axis.^{74,75}

In certain types of cell models, the HGF/c-MET signaling pathway is equivalent to the EMT.⁷⁶ The EMT or scattering is a

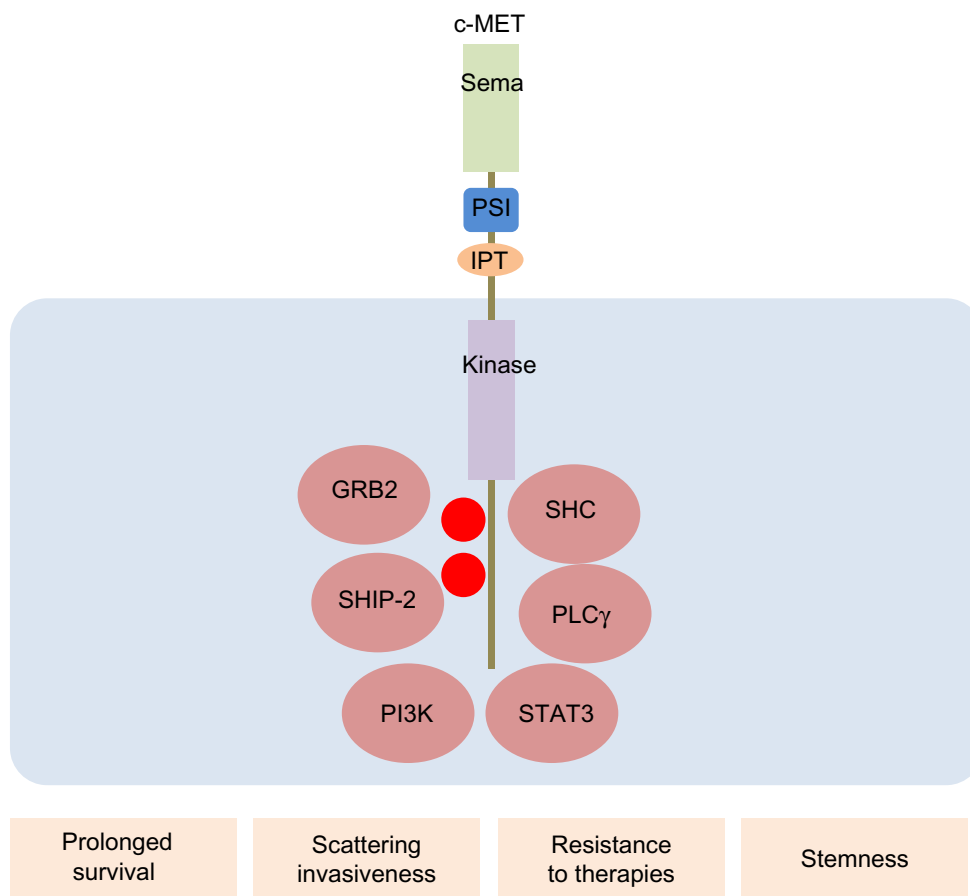


Figure 2 c-MET mediates EMT-promoting signals in cancers. The extracellular portion of c-MET is composed of three domains: the Sema, PSI, and IPT. The intracellular portion of c-MET contains a tyrosine kinase catalytic domain with flanked juxtamembrane and carboxy-terminal sequence. HGF binding to c-MET induces receptor homodimerization and phosphorylation of tyrosine residues (Y1234 and Y1235) within the catalytic loop, followed by phosphorylation of Y1349 and Y1356 in the carboxyl-terminal tail. When these tyrosine residues are phosphorylated, various adaptor and effector molecules, including growth factor receptor bound protein 2, Src homology-2-containing phosphatidylinositol 3-kinase, phospholipase C γ , Src homology domain-containing 5' inositol phosphatase, and STAT3, are recruited. These intracellular signaling molecules mediate EMT-related cellular responses that promote prolonged survival, scattering/invasiveness, resistance to conventional and molecular targeted therapies, and self-renewal/stemness in cancer cells.

Abbreviations: EMT, epithelial to mesenchymal transition; HGF, hepatocyte growth factor; IPT, immunoglobulin–plexin–transcription; PI3K, phosphoinositide 3-kinase; PSI, plexins, semasphorins, and integrins; STAT3, signal transducer and activator of transcription 3.

crucial event in the morphogenic processes of embryogenesis.⁷⁷ HGF and c-MET are coexpressed in progenitor cells during the early stage of mouse embryogenesis and are involved in the generation of endoderm and mesoderm during development of Hensen's node.⁷⁸ HGF promotes the EMT during somitogenesis and development of the endocardial cushion through the c-MET/Crk adaptor protein.⁷⁹ Paracrine HGF derived from mesenchymal cells stimulates epithelial and myoblast progenitors during organogenesis.⁷⁸ Axonal guidance of motor neurons by HGF results in innervations of striatal muscles, triggering coordinated development.⁸⁰

Canadas et al⁸¹ demonstrated that inhibition of MET signaling results in the reversal of biomarkers associated with the EMT and subsequently increases chemosensitivity in SCLC models. EMT-like phenotypes, including down-regulated E-cadherin and increased expression of Snail and N-cadherin, were observed in HGF-treated hepatocellular carcinoma cells. Sorafenib potently reverses the EMT process by inhibiting HGF-mediated MET and downstream kinases such as MAPK.⁸² Lu et al⁸³ demonstrated that inhibiting MET blocks the EMT and invasive growth in a GBM mouse model provoked by VEGF ablation (Figure 2).

MET signaling in cancers and GBM

MET signaling pathway in general cancers

Somatic mutations in *MET* are associated with tumor aggressiveness and metastasis in various human malignancies.⁸⁴ Cancer cells harboring *MET* mutations are observed particularly in the metastatic lymph node region in certain cases of head and neck cancer.⁸⁵ In addition, NIH 3T3 cells, which endogenously express HGF, become tumorigenic in nude mice when MET is overexpressed ectopically. Furthermore, cells co-overexpressing human MET and HGF metastasize to the lung and produce invasive tumors in the heart, diaphragm, and retroperitoneum.⁸⁶ The c-MET autocrine loop has been identified in cancer cells and is indicative of tumor aggressiveness and poor prognosis in NSCLC and breast cancer.^{87,88} MET amplification was detected in approximately 22% of lung cancer specimens that are resistant to EGFR inhibitors such as gefitinib and erlotinib. Ectopic expression of MET induces resistance to gefitinib in gefitinib-sensitive lung cancer cells. Moreover, MET knockdown restores sensitivity to gefitinib.⁸⁹

MET signaling pathway in GBM

The high expression level of HGF/SF and MET is significantly correlated with the tumor grade in human primary brain tumors. Moreover, HGF/MET signaling induces cell

proliferation and invasive growth in GBM cell lines but does not affect proliferation in normal human astrocytes.⁹⁰ Kong et al⁹¹ reported that MET overexpression is associated with shorter overall survival and poor treatment responses in GBM. They also found that MET expression is significantly associated with matrix metalloproteinase (MMP)-2 and -9, which are indicative of the EMT in cancer. Liu et al⁹² demonstrated that recurrent GBM expresses a higher level of MET than do primary tumors, and that MET overexpression is associated with shorter progression-free survival in patients with GBM. Activation of the HGF/MET axis has been detected in some human glioblastoma cell lines such as U-373 MG and T98G, and it promotes activation of the Ras, MAPK, AP-1, PI3K, and protein kinase C signaling pathways.⁹³ Also, MET signaling activation was detected in a GSC-rich fraction, and MET expression correlates with stem cell marker expression in GBM. Activating MET also inhibits the forced differentiation of GBM neurospheres.⁹⁴ Joo et al¹⁹ found that a distinct fraction of MET overexpressing cells from patient-derived GSCs were highly clonogenic, tumorigenic, and resistant to radiation. Furthermore, inhibiting MET signaling resulted in disrupted tumorigenicity and invasive growth in GSCs derived from patient tumor specimens. One study reported that HGF/MET signaling induces invasive tumor growth through direct activation of Wnt/ β -catenin signaling in GSCs.⁹⁵

Targeting MET signaling in cancers and GBM

Targeting the MET signaling pathway in general cancers

NK4, a four-kringle domain containing intracellular fragment of HGF, was identified as a competitive inhibitor of HGF/MET signaling via suppression of the specific binding of HGF to its receptor (Figure 3 and Table 2).⁹⁶ Administering NK4 by gene therapy suppresses tumor invasion and metastasis in colon cancer cells by inactivating the HGF/MET signaling pathway.⁹⁷ An engineered soluble MET receptor, decoy MET, interferes with HGF binding to MET and MET homodimerization.⁹⁸ The decoy MET inhibits cell proliferation, angiogenesis, and spontaneous metastasis in various cancer cells.

AMG 102, a neutralizing, fully human monoclonal antibody against human and nonhuman primate HGF, inhibited HGF-induced c-MET autophosphorylation in PC3 cells and suppressed HGF-induced migration of human MDA-MB-435 cells (Figure 3).⁹⁹

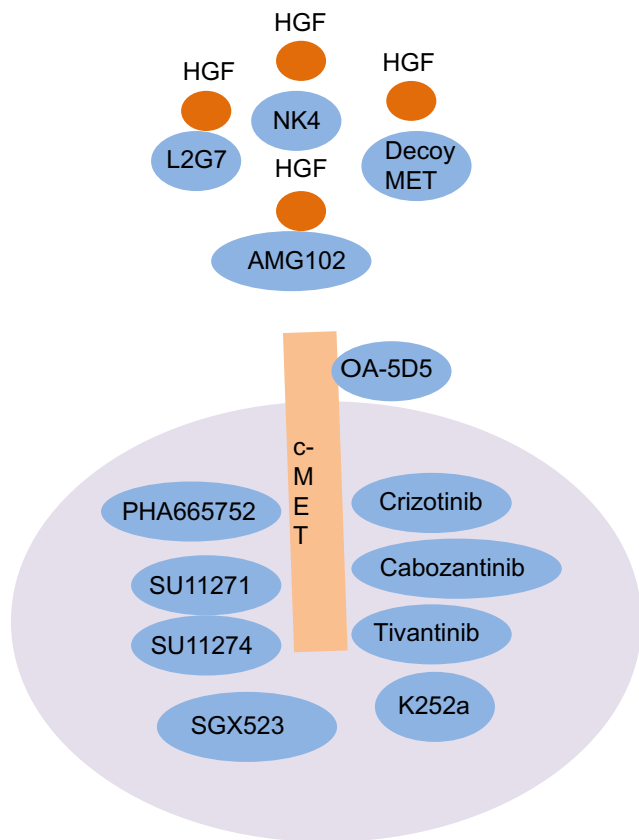


Figure 3 Current therapeutics targeting the HGF/MET signaling pathway. NK4, a four-kringles-containing intracellular fragment of HGF, was identified as a competitive inhibitor of HGF/MET signaling through suppression of specific binding of HGF to the receptor. An engineered soluble MET receptor, decoy MET, interferes with HGF binding to MET. L2G7 is an anti-HGF monoclonal antibody that suppresses tumor growth by inhibition of the MET signaling pathway. A neutralizing, fully human monoclonal antibody against human and nonhuman primate HGF, AMG 102, inhibited HGF-induced c-MET autophosphorylation. A novel OA-5D5 anti-c-MET antibody suppresses the growth of U87MG cells. An adenosine triphosphate analog, K252a, potentially inhibits HGF-mediated morphology changes and suppresses MET-mediated proliferation. A selective small molecular inhibitor, PHA-665752, inhibits the catalytic activity of c-MET and downstream kinases. PHA-665752 also suppresses the HGF-driven cell growth, motility, and morphology changes. SU11271, a potent and selective pyrrole-indolinone inhibitor of MET tyrosine kinase, suppresses HGF-mediated cell proliferation, survival, and invasion in various cancer cells. SU11274 reduces the activity of constitutively active TPR-MET fusion protein, followed by inducing apoptosis in cells transformed by the oncogenic TPR-MET. Crizotinib, a dual inhibitor of MET and ALK tyrosine kinases, inhibits cells dependent upon c-MET or ALK activity. Cabozantinib, a multitargeted tyrosine kinase inhibitor, suppresses the activity of MET and vascular endothelial growth factor receptor 2. Tivantinib, a potent small molecular inhibitor of c-MET, induces cell-cycle arrest and inhibits the invasive growth of various cancer cells.

Abbreviations: ALK, anaplastic lymphoid kinase; HGF, hepatocyte growth factor; OA-5D5, one-armed 5D5.

The adenosine triphosphate analog K252a potentially inhibited HGF-mediated morphological changes in MLP-29 cells and suppressed MET-mediated proliferation in GTL-16 gastric carcinoma cells. Moreover, administering K252a inhibited metastasis to the lungs following injection of GTL-16 cells into the caudal vein of a mouse metastasis model.¹⁰⁰ The selective small molecular inhibitor PHA-665752, which inhibits c-MET catalytic activity, potentially inhibits HGF-induced

c-MET phosphorylation and downstream kinases, including extracellular regulated kinase, Akt, and STAT3. PHA-665752 also suppressed HGF-driven cell growth, motility, and morphological changes.¹⁰¹ PHA-665752 specifically triggered apoptosis in gastric cancer cells with MET amplification. SU11271, a potent and selective pyrrole-indolinone inhibitor of MET tyrosine kinase, suppresses HGF-mediated cell proliferation, survival, and invasion in various cancer cells.¹⁰² SU11274, another inhibitor of MET kinase activity, reduced the activity of the constitutively active TPR-MET fusion protein, followed by induction of apoptosis in cells transformed with the oncogenic TPR-MET.¹⁰³ SU11274 also inhibits phosphorylation of c-MET and downstream kinases, including Akt, in NSCLC cells (Figure 3).¹⁰⁴

Crizotinib, a dual inhibitor of MET and ALK tyrosine kinases, inhibits cells dependent upon c-MET or ALK activity.¹⁰⁵ In addition, crizotinib inhibits the growth and metastasis of uveal melanoma cells by inactivating MET in a metastatic melanoma mouse model.¹⁰⁶ Cabozantinib, a multitargeted tyrosine kinase inhibitor of MET and vascular endothelial growth factor receptor 2, reduces cell growth and invasiveness in vitro, as well as bone metastasis of prostate cancer cells in vivo, by inhibiting the MET signaling pathway.¹⁰⁷ A potent small molecule inhibitor of c-MET, tivantinib, induces cell-cycle arrest and reduces cell viability and invasive growth in various cancer cells (Figure 3).¹⁰⁸

Targeting the MET signaling pathway in GBM

Neutralizing monoclonal antibodies potentially inhibit growth of xenograft tumors from human GBM cells expressing MET and HGF.¹⁰⁹ Additionally, the anti-HGF L2G7 monoclonal antibody is capable of suppressing tumor growth by inhibiting the MET signaling pathway. Systemic administration of L2G7 significantly prolongs the median survival of mice with intracranial tumors.¹¹⁰ An anti-HGF antibody manufactured using human–mouse chimeric HGF proteins inhibits HGF-dependent tumor growth and suppresses tumor growth in a xenograft model of the U87MG glioma cell line.¹¹¹ A novel one-armed 5D5 (OA-5D5) anti-c-MET antibody suppresses growth of U87MG cells by >95%. In addition, treatment with OA-5D5 downregulates the expression of urokinase-type plasminogen activator and MMP-16 in GBM cells.¹¹²

The MET kinase inhibitor SGX523 inhibits in vivo tumor growth dramatically in GBM cell lines such as U87M2, U118, and SF295SQ1.¹¹³ Xie et al¹¹³ demonstrated that activation of HGF autocrine mechanism and serum HGF levels in

Table 2 Summary of targeted therapies for MET signaling

Agents	Mechanism of action	Cancer type	In vivo or vitro	Effect and efficacy	Reference
NK4	Suppression of HGF binding to MET	Colon cancer	In vivo	Suppresses tumor invasion and metastasis	96,97
Decoy MET	Suppression of HGF binding to MET. Inhibition of MET homodimerization	Melanoma	In vitro, in vivo	Inhibits proliferation and survival and suppresses angiogenesis and metastasis	98
AMG 102	mAb to HGF	Prostate cancer, melanoma	In vitro	Inhibits MET phosphorylation and suppresses migration	99
L2G7	mAb to HGF	Glioblastoma	In vitro, in vivo	Suppresses tumor growth, prolongs median survival, and decreases stem cell markers	111,115
OA-5D5	Anti-cMET antibody	Glioblastoma	In vitro	Suppresses growth and inhibits invasiveness-related markers	112
K252a	ATP analog	Liver cells, gastric cancer	In vitro, in vivo	Inhibits HGF-mediated morphologic changes and suppresses lung metastasis	100
PHA-665752	Inhibits the catalytic activity of c-MET	Gastric cancer	In vitro, in vivo	Inhibits HGF-induced MET phosphorylation and downstream kinases; suppresses HGF-driven cell growth, motility, and morphologic change; and triggers apoptosis	101
SU11271	A selective pyrrole-indolinone inhibitor of MET tyrosine kinase	Lung cancer, prostate cancer, kidney cells	In vitro	Suppresses HGF-dependent cell growth, survival, and invasion	102
SU11274	A selective pyrrole-indolinone inhibitor of MET tyrosine kinase	Lung cancer	In vitro	Reduces the activity of constitutively active MET and suppresses the activities of downstream kinases of MET	103,104
SGX523	MET kinase inhibitor	Glioblastoma	In vitro, in vivo	Inhibits tumor growth, decreases stem cell markers, and synergizes the efficacy of EGFR inhibitors	113,114
Crizotinib (Xalkori®)	Dual inhibitor of MET and ALK	Melanoma, glioblastoma	In vitro, in vivo	Inhibits MET phosphorylation, suppresses the tumor growth and metastasis, and decreases stem cell markers	105,106
Cabozantinib (Cometriq™)	A multikinase inhibitor of MET and VEGFR2	Prostate cancer	In vitro, in vivo	Reduces cell growth and invasiveness and inhibits bone metastasis	107
Tivantinib (ARQ197)	A staurosporine derivative and a selective MET inhibitor of unknown mechanism	Liver cancer, ovarian cancer, gastric cancer	In vitro	Reduces cell survival and invasive growth	108

Abbreviations: ALK, anaplastic lymphoid kinase; ATP, adenosine triphosphate; HGF, hepatocyte growth factor; mAb, monoclonal antibody; OA-5D5, one-armed 5D5; VEGFR2, vascular endothelial growth factor receptor 2.

a xenograft model, rather than MET amplification or HGF paracrine effects, serve as biomarkers for predicting sensitivity to MET inhibition in GBM cell lines. A combination of SGX523 and erlotinib, an EGFR inhibitor, synergistically decreased tumor growth in U87M2 cells.¹¹⁴

Inhibiting the MET pathway by neutralizing the anti-HGF monoclonal antibody L2G7 or crizotinib potentially decreased tumor growth and the expression of stem cell markers such as CD133, Sox2, Nanog, and Musashi in a pre-established GBM xenograft model.¹¹⁵ Furthermore, serial transplantation of xenograft-derived cells from mice administered c-MET inhibition therapy resulted in depleted tumor formation ability and smaller tumor size, compared with control mice.¹¹⁵ L2G7 also synergized the antitumor efficacy of erlotinib against a PTEN-null/EGFRvIII+ GBM xenograft model.¹¹⁶

These results support that inhibiting MET signaling alone may diminish tumor growth in certain GBM cell types, and

also that MET inhibitors may synergize the effect of EGFR inhibitors in GBM cells with EGFR vIII expression and PTEN deletion.

Conclusion

The EMT has emerged as a critical event in tumor invasion and metastasis. The EMT also plays a pivotal role in tumor aggressiveness and the development of therapeutic resistance in GBM patients. Activating the HGF/MET signaling pathway is indicative of the EMT because HGF potentially induces cell scattering.

Preclinical and clinical trials to inhibit MET signaling by neutralizing human HGF, antagonizing the MET receptor, and inhibiting MET enzymatic activity have progressed. Several monoclonal antibodies and receptor tyrosine kinase inhibitors have been tested clinically for treatment of advanced/metastatic cancer.

Several issues should be considered to maximize the effect of MET inhibition therapy. First, selecting the target population selection is crucial because MET inhibition is often insufficient to suppress the invasive growth of tumors with normal MET expression. Tumors harboring amplified MET and activating MET mutations could be an initial selection strategy for establishing a target population for MET inhibition therapy. Another target population of interest could be those patients administered antiangiogenic therapy with VEGF ablation. Because VEGF ablation may induce invasive growth by rebound activation of the MET signaling pathway,⁸³ MET inhibition could suppress the emerging outgrowth of cells induced by antiangiogenic therapy. Second, considering a combination of chemotherapeutics or small molecule inhibitors of other major growth pathways would be beneficial, since inhibition of MET signaling alone may be insufficient to induce massive apoptosis or reduce tumor volume. Inhibiting other major growth pathways, including EGFR or FGFR, in combination with HGF/MET, may trigger effective reductions in tumor burden as well as invasive growth in certain cancer cases.

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Disclosure

The authors report no conflicts of interest in this work.

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