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The hepatocyte growth factor/c-Met signaling pathway as a therapeutic target to inhibit angiogenesis

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Abstract

Angiogenesis in tumors is driven by multiple growth factors that activate receptor tyrosine kinases. An important driving force of angiogenesis in solid tumors is signaling through vascular endothelial growth factor (VEGF) and its receptors (VEGFRs). Angiogenesis inhibitors that target this signaling pathway are now in widespread use for the treatment of cancer. However, when used alone, inhibitors of VEGF/VEGFR signaling do not destroy all blood vessels in tumors and do not slow the growth of most human cancers. VEGF/VEGFR signaling inhibitors are, therefore, used in combination with chemotherapeutic agents or radiation therapy. Additional targets for inhibiting angiogenesis would be useful for more efficacious treatment of cancer. One promising target is the signaling pathway of hepatocyte growth factor (HGF) and its receptor (HGFR, also known as c-Met), which plays important roles in angiogenesis and tumor growth. Inhibitors of this signaling pathway have been shown to inhibit angiogenesis in multiple *in vitro* and *in vivo* models. The HGF/c-Met signaling pathway is now recognized as a promising target in cancer by inhibiting angiogenesis, tumor growth, invasion, and metastasis.

Keywords

Angiogenesis inhibitors; Endothelial cells; HGFR; Receptor tyrosine kinases; Signal transduction; Tumors; VEGF; VEGFR

INTRODUCTION

Angiogenesis, the formation of new blood vessels from the existing vasculature, contributes to many diseases, including cancer, age-related macular degeneration, diabetic retinopathy, neovascular glaucoma, psoriasis, and rheumatoid arthritis (1, 2). In solid tumors, angiogenesis is driven by multiple growth factors that act on via receptor tyrosine kinases (1, 2). An important driving force of angiogenesis in solid tumors is the signaling pathway of vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) (2).

Several angiogenesis inhibitors that target the VEGF/VEGFR signaling pathway have been approved by the FDA (Food and Drug Administration) and are now used for the treatment of cancer patients (3–6). The first approved inhibitor of this signaling pathway is bevacizumab

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(Avastin, from Genentech), which is a monoclonal antibody against human VEGF (3). The other inhibitors are sunitinib (SU11248, from Pfizer) (4, 5) and sorafenib (BAY 43-9006, from Bayer) (6), which are small molecule compounds that inhibit phosphorylation of VEGFR and certain other receptor tyrosine kinases.

VEGF/VEGFR signaling inhibitors can block VEGF-driven angiogenesis in tumor models in mice (7, 8). These inhibitors cause regression of tumor vessels that depend on VEGF as a survival factor. However, tumor vessels that do not regress after VEGF signaling inhibition tend to become more normal ("normalization") (8–11). Moreover, VEGF signaling inhibitors alone do not destroy all blood vessels in tumors (12). Therefore, additional targets of angiogenesis are being sought to augment the effects of VEGF inhibitors (13).

One promising target is the signaling pathway of hepatocyte growth factor (HGF, also known as scatter factor, SF) along with its receptor (HGFR, also known as c-Met). HGF, a potent mitogenic, motogenic and morphogenic factor that also plays an important role in angiogenesis and tumor growth (14–16). HGF and VEGF act synergistically on endothelial cells (16–19). HGF and c-Met are upregulated in many human cancers (20–22). Activation or upregulation of c-Met is a negative prognostic indicator in patients with various carcinomas, multiple myeloma, or glioma (23–26). For these reasons, various inhibitors of HGF/c-Met signaling pathway are being studied and developed as additional potent therapies to inhibit angiogenesis and tumor growth.

Molecular structure of HGF and c-Met

HGF is a multifunctional growth factor (20–22). It is produced as a single-chain inactive precursor protein (27, 28) (Fig. 1A). Mature active HGF is a heterodimer composed of an alpha- chain subunit (69 kDa) and a beta-chain subunit (34 kDa), which are linked by a disulfide bond (27, 29). The alpha-chain subunit contains an N-terminal hairpin domain and four kringle domains; the beta-chain subunit is a serine-protease-like domain lacking catalytic activity due to mutations in essential residues (27).

c-Met is also produced as a single-chain precursor protein (30, 31). This precursor receptor is cleaved to produce a glycosylated alpha-chain subunit (50 kDa) and a transmembrane beta- chain subunit (145 kDa), which are linked by a disulfide bond to form the mature receptor (32) (Fig. 1B).

The extracellular portion of c-Met, which is responsible for binding to HGF, contains a Sema domain (homologous to semaphorins), a cysteine-rich Met-related-sequence (MRS) domain, and four immunoglobulin-like structure (IgG domain) (32).

The intracellular portion of c-Met, which is responsible for signal transduction, is composed of a juxtamembrane domain, a tyrosine kinase domain, and a C-terminal regulatory tail (33). The juxtamembrane domain plays a key role in downregulation of the receptor (34, 35). The phosphorylation of a serine residue (Ser 985) in this domain inhibits the tyrosine kinase activity of c-Met (34). In addition, the phosphorylation of a tyrosine residue (Tyr 1003) is responsible for polyubiquitination and degradation of the receptor (35).

In the tyrosine kinase domain, two tyrosine residues (Tyr 1234 and Tyr 1235) regulate the kinase activity of c-Met (36). The other two tyrosine residues (Tyr 1349 and Tyr 1356), which are located in the C-terminal regulatory tail (the multi-substrate docking site), are the most important sites for recruiting downstream adapter molecules (37–39). Both tyrosine residues in the C-terminal tail are sufficient for the signal transduction of c-Met *in vitro* and *in vivo* (38, 40).

Expression of HGF and c-Met

HGF is expressed only by cells of mesenchymal origin (41). However, c-Met is expressed mainly by epithelial cells (41). In addition, c-Met is expressed by various other cell types including vascular endothelial cells (16), lymphatic endothelial cells (42), neural cells (43), hepatocytes (44), hematopoietic cells (45), and pericytes (46). In many tumor cells, c-Met expression is activated by HGF through an autocrine loop (47–52). The activation or upregulation of both the ligand and the receptor in tumors is a negative prognostic indicator in human cancer (23–26, 53, 54).

HGF/c-Met signaling pathway in angiogenesis

The HGF/c-Met signaling pathway plays an important role not only in embryogenesis and development but also in angiogenesis and tumor growth (15, 16, 19–22). This multifunctional signaling pathway induces mitogenesis, motogenesis, morphogenesis and angiogenesis (20–22) (Fig. 2).

On the molecular level, after ligand binding, c-Met is activated by phosphorylation of Tyr 1234 and Tyr 1235 residues, located in the tyrosine kinase domain (36). The phosphorylation of the other two tyrosines (Tyr 1349 and Tyr 1356), located in the C-terminal tail, provides a docking site for multiple substrates of downstream signal transduction such as Src, Gab1, and Grb2 (37). Therefore, HGF/c-Met signaling activates multiple signal transduction pathways including the Src/focal adhesion kinase (FAK) pathway, the p120/signal transducer and activator of transcription (STAT) 3 pathway, the phosphoinositide- 3 kinase (PI3K)/Akt pathway, and the Ras/MEK pathway (38, 39). The Src/FAK pathway regulates cell adhesion and migration (20–22). The p120/STAT3 pathway stimulates branching morphogenesis of cells (20–22). The PI3K/Akt pathway activates cell motility and cell survival (20–22). The Ras/MEK pathway mediates HGF-induced scattering and proliferation of cells (20–22). Thus, these multiple signaling pathways directly or indirectly stimulate endothelial cells: directly by motogenic or morphogenic effects and indirectly by regulation of other angiogenic factors (17–19). HGF increases expression of angiogenic mediators, including VEGF and its receptor, in endothelial cells (17).

Development of inhibitors targeting HGF/c-Met signaling pathway

Because HGF/c-Met signaling is activated in angiogenesis and tumor growth, several strategies have been explored for inhibiting the pathway (20–22). The strategies are based on the lessons learned from studies on development of inhibitors targeting other ligands and receptor tyrosine kinases (3–5, 55). Each strategy targets one of the molecular events of HGF/c-Met activation (Fig. 2). As seen in other signal transduction pathways of receptor tyrosine kinases, HGF binds to its receptor, c-Met, on the cell surface, and then the tyrosine

kinase domain of c-Met is activated by dimerization and transphosphorylation (20–22, 56). The activation of these catalytic tyrosine residues is followed by additional phosphorylation of the two tyrosines in the C-terminal regulatory tail (20–22). This fully active receptor is ready to propagate c-Met-dependent signals by recruiting and stimulating downstream signaling molecules (20–22).

One strategy for inhibiting HGF/c-Met signaling is to block the binding of HGF to c-Met (Fig. 2A). Inhibitors of HGF/c-Met binding include HGF antagonists and antibodies against HGF or c-Met (Table 1). One HGF antagonist, NK4, is a truncated form of HGF, which contains the N-terminal hairpin domain and the subsequent four kringle domains (57, 58). NK4 binds to c-Met without activating it (57). The action of NK4, which has been studied in multiple *in vitro* and *in vivo* models using different approaches of delivery, is the best-characterized competitive antagonist of HGF (57, 58). Recently, other antagonists of HGF/c-Met binding have been developed, including an uncleavable HGF (59), an N-terminal Sema domain of HGF (60), a soluble extracellular domain of c-Met (decoy Met) (61), and a recombinant splice variant of c-Met (62). In addition, an antibody against HGF or c-Met inhibits angiogenesis and tumor growth in tumor models by blocking the binding of HGF and c-Met (63–67).

Another strategy is targeting phosphorylation of the tyrosine residues in the tyrosine kinase domain of c-Met (Fig. 2B, Table 2). Most common agents in this group are small molecule inhibitors of c-Met receptor tyrosine kinase. Most of them are competitive analogues of ATP, a substrate for phosphorylation of c-Met tyrosine kinase. Several small molecule c-Met inhibitors are being studied and developed (68–73). In contrast to more specific inhibition by inhibitors of HGF/c-Met binding, small molecule inhibitors may have broader specificity for targeting receptor tyrosine kinases. However, most small molecule inhibitors can be administered orally for treatment of cancer patients.

The third strategy is to inhibit signaling events downstream of the HGF/c-Met signaling pathway (21, 22) (Fig. 2C, Table 2). A selective PI3K inhibitor, LY294002, inhibits HGF/c-Met-induced cell motility and morphogenic changes (74). A MEK inhibitor, PD98059, prevents invasiveness of malignant tumor cells dependent on HGF/c-Met signaling (75). Src inhibitors, PD180970 and SU6656, reduce Src and STAT3 activity in lung cancer cells stimulated by HGF (76).

Conclusion and perspectives

Recently, the HGF/c-Met signaling pathway has come into the spotlight as a promising therapeutic target for inhibiting angiogenesis. Research over the past two decades has revealed that the HGF/c-Met signaling pathway plays an important role in angiogenesis and tumor growth, that this signaling pathway acts on angiogenesis synergistically with the VEGF/VEGFR signaling pathway, and that the HGF/c-Met signaling pathway promotes tumor invasion and metastasis. Inhibitors of HGF/c- Met signaling, used in combination with inhibitors of VEGF/ VEGFR signaling, should have greater efficacy in slowing angiogenesis and tumor growth and perhaps reducing tumor invasion and metastasis.

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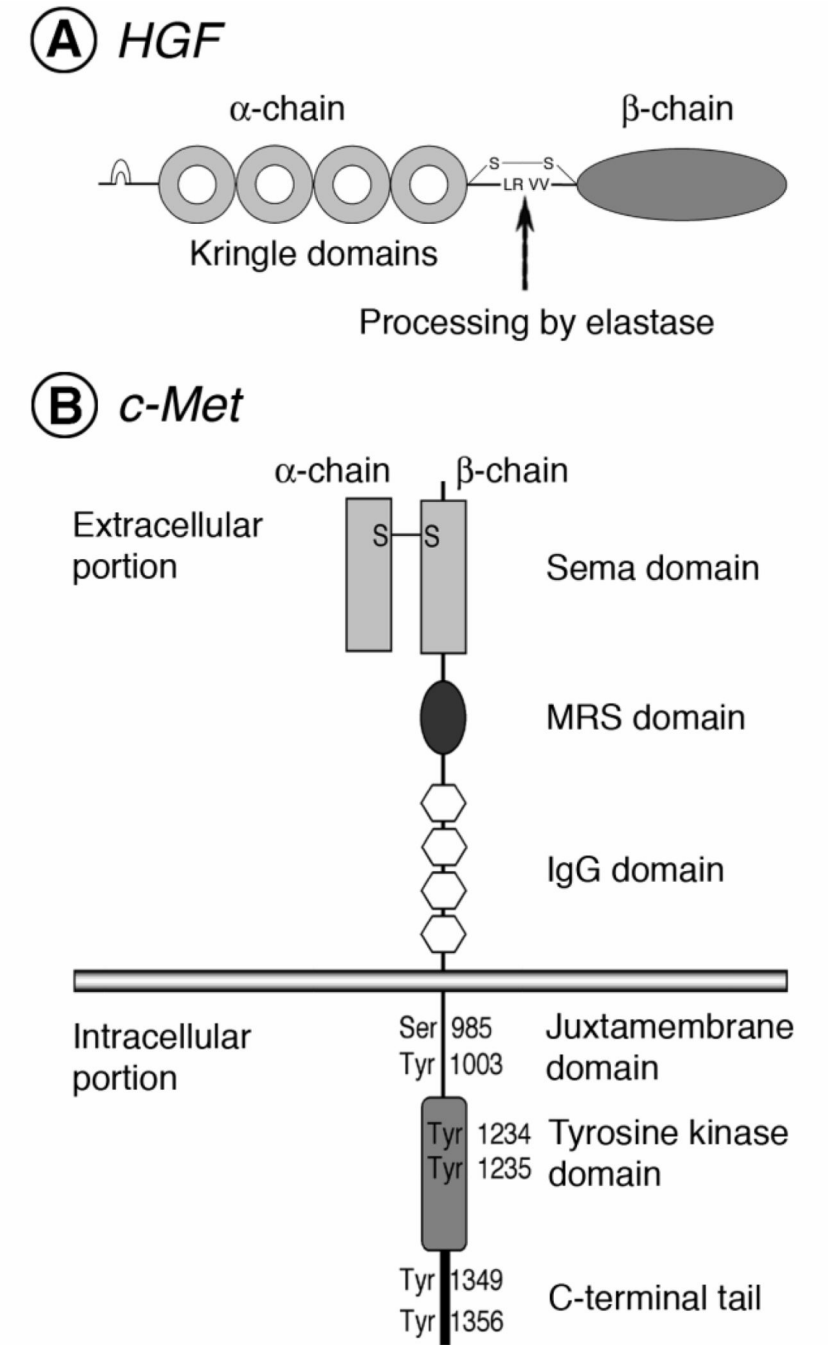

Fig. 1.

Schematic molecular structure of HGF and its receptor, c-Met. HGF (A) and c-Met (B) are initially expressed as precursor proteins and are then cleaved to mature heterodimers composed of an alpha- chain subunit and a beta-chain subunit linked by a disulfide bond. Mature HGF is a heterodimer composed of an alpha chain, which contains an N-terminal hairpin domain and four kringle domains, and a beta chain consisting of a serine-protease-like domain without enzymatic activity. Mature c-Met is composed of a glycosylated alpha subunit and a transmembrane beta subunit. The extracellular region of mature c-Met

contains a Sema domain, a cysteine-rich Met-related sequence (MRS) domain, and four immunoglobulin-like structure domain. The intracellular region is composed of a juxtamembrane domain, a tyrosine kinase domain, and a C-terminal regulatory tail.

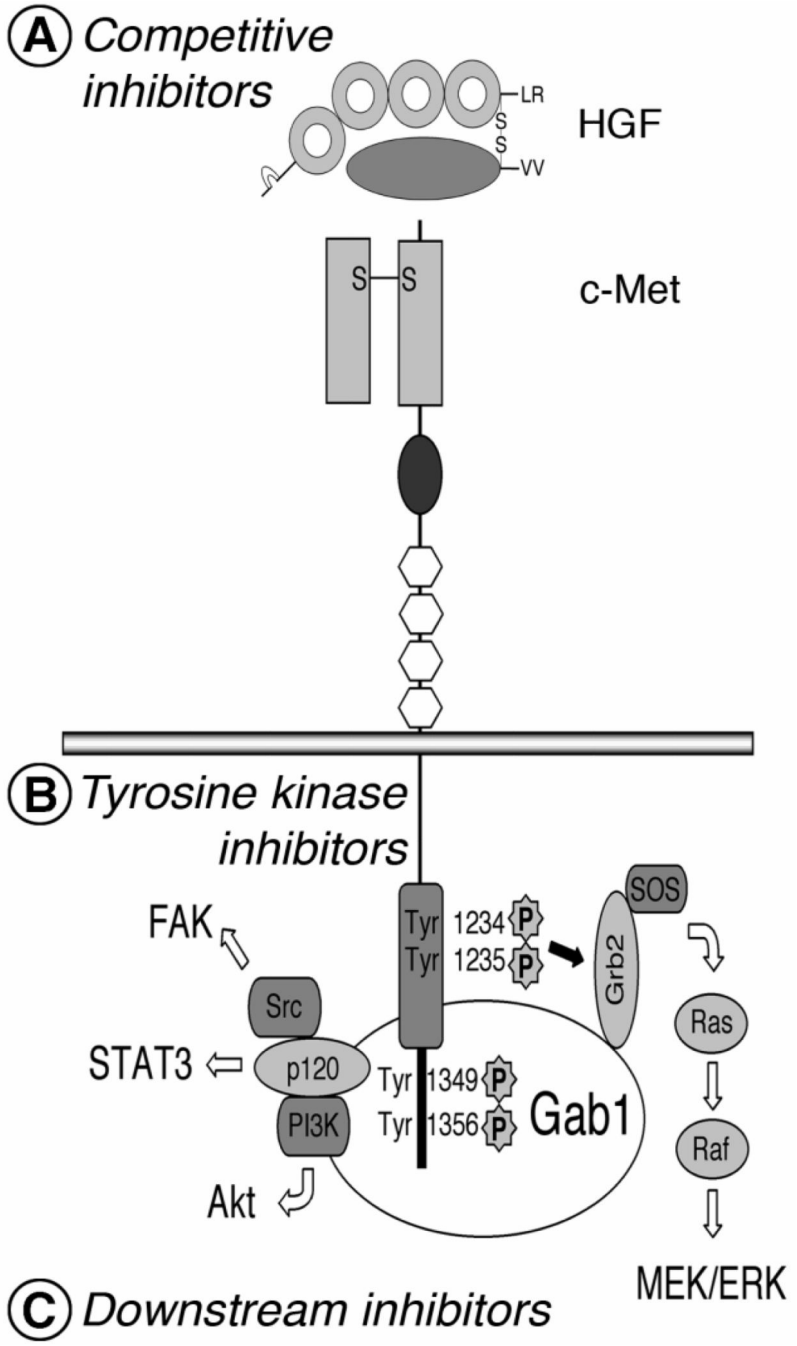

Fig. 2.

Summary of the HGF/c-Met signaling pathway. HGF/c-Met signal transduction is initiated by binding of HGF to c-Met, as with other receptor tyrosine kinases. Dimerization or oligomerization of c-Met activates transphosphorylation of tyrosines (Tyr1234 and Tyr 1235) in the kinase domain followed by additional phosphorylation of other tyrosines (Tyr 1349 and Tyr 1356) in the C-terminal regulatory tail. Fully activated c-Met propagates HGF signaling in cells by recruiting and activating various adapter molecules downstream. Inhibitors of the HGF/c-Met signaling pathway, competitive inhibitors (A), tyrosine kinase

inhibitors (B) or downstream inhibitors (C), target one of the molecular events of HGF/c-Met signaling activation and transduction.

Table 1

Antagonists of HGF and c-Met binding

Group	Compound	Developmental stage	References
HGF/c-Met antagonists	NK4	Preclinical	(57, 58)
	Uncleavable HGF	Preclinical	(59)
	Sema	Preclinical	(60)
	Decoy Met	Preclinical	(61)
	Recombinant variant Met	Preclinical	(62)
Antibodies against HGF	L2G7 (Galaxy Biotech)	Preclinical	(63)
	AMG102 (Amgen)	Phase II	(64)
Antibodies against HGF	OA-5D5 (Genentech)	Preclinical	(65)
	CE-355621 (Pfizer)	Preclinical	(66)
	DN30 (Metheresis)	Preclinical	(67)

Table 2

Inhibitors of the HGF/c-Met signaling pathway

Group	Compound	Developmental stage	References
Small molecule inhibitors of c-Met tyrosine kinase	SU11274 (Pfizer)	Preclinical	(68)
	PHA665752 (Pfizer)	Preclinical	(69)
	PF2341066 (Pfizer)	Phase I/II	(70)
	XL880 (Exelixis)	Phase II	(71, 72)
	XL184 (Exelixis)	Phase III	(73)
Downstream inhibitors	LY294002 (LC Laboratories)	Preclinical	(74)
	PD98059 (LC Laboratories)	Preclinical	(75)
	PD180970 (Parke-Davis Pharmaceutical Research)	Preclinical	(76)
	SU6656 (Pfizer)	Preclinical	(76)