The role of the c-Met pathway in lung cancer and the potential for targeted therapy

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Abstract: Hepatocyte growth factor receptor (HGFR), the product of the *MET* gene, plays an important role in normal cellular function and oncogenesis. In cancer, HGFR has been implicated in cellular proliferation, cell survival, invasion, cell motility, metastasis and angiogenesis. Activation of HGFR can occur through binding to its ligand, hepatocyte growth factor (HGF), overexpression/amplification, mutation, and/or decreased degradation. Amplification of HGFR can occur *de novo* or in resistance to therapy. Mutations of HGFR have been described in the tyrosine kinase domain, juxtamembrane domain, or semaphorin domain in a number of tumors. These mutations appear to have gain of function, and also reflect differential sensitivity to therapeutic inhibition. There have been various drugs developed to target HGFR, including antibodies to HGFR/HGF, small-molecule inhibitors against the tyrosine kinase domain of HGFR and downstream targets. Different HGFR inhibitors are currently in clinical trials in lung cancer and a number of solid tumors. Several phase I trials have already been completed, and two specific trials have been reported combining HGFR with epidermal growth factor receptor (EGFR) inhibition in non-small cell lung cancer. In particular, trials involving MetMAb and ARQ197 (tivantinib) have gained interest. Ultimately, as individualized therapies become a reality for cancers, HGFR will be an important molecular target.

Keywords: hepatocyte growth factor, kinase inhibitors, MET, targeted therapy

Biology of lung cancer

The most common cancer to be diagnosed in the United States is lung cancer. There are estimated an 222,520 cases diagnosed in 2010. The mortality burden from this disease is quite high, with an estimated 157,300 deaths expected in that year [Jemal *et al.* 2010]. According to Surveillance, Epidemiology, and End Results (SEER) 17 data, the age-adjusted incidence of cancer of the lung and bronchus was 62.5 per 100,000 persons per year from 2003 to 2007. There are a large number of molecular and cellular abnormalities that can occur in lung cancer [Salgia and Skarin, 1998]. As an example, there can be oncogenic activation of receptor tyrosine kinases, activation of signal transduction pathways, and deletion of tumor suppressors such as p53 and Rb. One of the emerging themes in lung cancer is the alteration of receptor tyrosine kinases (RTKs). Over the past several years, the epidermal growth factor receptor (EGFR) has been shown to be mutated (in particular the tyrosine kinase domain), with mutation being predictive of therapeutic response to small-molecule

inhibition with agents such as erlotinib and gefitinib. Another RTK that can be genetically altered is ALK on chromosome 2. A translocation on chromosome 2p results in the generation of the potent oncogene EML4-ALK. This fusion protein has recently been shown to be a target of the small-molecule inhibitor, crizotinib (formerly PF02341066). In this review, we focus on the RTK MET. MET RTK has been shown to be important in normal homeostasis, and signal transduction, and is abnormal in various cancers.

The hepatocyte growth factor receptor is expressed on functionally different cells

There is a considerable amount of systems biology established as related to hepatocyte growth factor (HGF) and its receptor (HGFR). The biological activities triggered by binding of HGF to HGFR, the product of the *MET* proto-oncogene, initiates a complex variety of responses. This is in part due to the expression of HGFR on functionally different cells (Table 1); its functions may include growth, transformation, cell motility, invasion, metastasis, epithelial to

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Table 1. Frequency of abnormalities in the hepatocyte growth factor receptor (HGFR)/MET pathway. The frequency of increased HGFR protein expression or mutation and amplification of MET is thought to be dependent on environmental factors and genetic predisposition. Alteration in the HGFR/MET pathway can ultimately lead to transformation of normal cells. All values are indicated as percentage of total (M = marginal).

Tumor type	HGFR expression	MET mutation	MET amplification
Brain	54–88	0–9	9–20
Breast	25–60	M	—
Cervical	30–72	0	—
Colon	55–78	0	4–89
Gastric	75–90	M	5–10
Head and neck	52–68	11–27	—
Hepatoma	68–69	0–30	—
Lung (NSCLC)	41–72	8–13	5–10
Melanoma	17–39	M	—
Mesothelioma	74–100	0	—
Multiple myeloma	48–80	—	—
Ovarian	64	0–4	0
Renal cell	54–87	13–100	(Trisomy 7)
Sarcoma	20–87	0–3	—
Thyroid	40–91	6–10	—

NSCLC, non-small cell lung cancer.

mesenchymal transition (EMT), angiogenesis, wound healing, or tissue regeneration depending on the cellular context [Weidner *et al.* 1996; Jeffers *et al.* 1996; Rong *et al.* 1994; Grant *et al.* 1993; Montesano *et al.* 1991; Stoker *et al.* 1987]. These broad biological functions are reflected in mice with *MET* gene disruption, which results in embryonic lethality with severe defects in liver and placenta development [Uehara *et al.* 1995]; the phenotype is similar in mice with a disruption of the *HGF* gene [Schmidt *et al.* 1995]. Further, both genetic models implicate HGF and HGFR in a broader role in morphogenesis and growth of multiple embryonic tissues, including the nervous system [Maina and Klein, 1999; Bladt *et al.* 1995; Schmidt *et al.* 1995; Uehara *et al.* 1995].

In addition to the induction of cell growth and reduced apoptosis, there are profound biological effects of activated HGFR on cytoskeletal function. The ability of activated HGFR to induce growth and scattering of epithelial cells has been well documented [Stoker *et al.* 1987]. Cell scattering is a process regulated through the cytoskeleton that involves cell migration, cell–cell dissociation, and cell spreading. For example, in a small-cell lung cancer (SCLC) model, stimulation of HGFR with HGF enhances cell motility, a process that involves the formation and retraction of filopodia/lamellipodia, changes in actin filament formation, and cell migration [Maulik

et al. 2002]. However, altered motility can also be induced by mutationally activated HGFR signaling in epithelial cells [Jeffers *et al.* 1998]. Progress has been made in understanding the signaling mechanisms by which HGF stimulation of HGFR leads to increased motility, migration, and invasion. A major downstream molecule is the phosphatidylinositol-3'-kinase, which is required for HGF-induced mitogenesis, morphogenesis, or morphogenesis in renal epithelial cells. Inhibition of its activity decreased branching formation on collagen and chemotaxis [Derman *et al.* 1996]. Also of interest is the focal adhesion protein paxillin, which is phosphorylated (pY31) in response to HGFR stimulation [Maulik *et al.* 2002]. Paxillin is highly overexpressed in lung cancers, in particular in metastatic tumors and frequently along with HGFR and EGFR. It is also mutated in almost 10% of all lung cancer cases [Jagadeeswaran *et al.* 2008]. The exact role of paxillin in HGFR transformation is not known, but activating mutations of HGFR (R988C and T1010I) in SCLC that modulate cellular transformation, anchorage-dependent proliferation, cytoskeletal motility and migration, also increase tyrosine phosphorylation of paxillin (pY31) [Ma *et al.* 2003].

Owing to the pleiotropic effects regulated through activated HGFR and the variety of cells that express the receptor, it is not surprising that this pathway has gained interest as a driving force

in tumor initiation, maintenance, and progression of not only lung cancer but also other malignancies. Additional efforts are being made to further understand the effectors of HGFR signaling and to evaluate its potential for targeted therapies. Initial clinical trials with various inhibitors of HGFR activation hold promise but the value of the activation and mutational status of MET as prognostic factors needs further assessment. It is not known whether HGFR cooperates with additional oncoproteins, or if the oncogenic potential of HGFR is increased by the deregulation of other signaling pathways.

Structure and function of HGFR and HGF

The human *MET* gene is located on chromosome 7 (7q31) and encodes a transmembrane protein. The first observation of MET as a potential proto-oncogene stems from its discovery as the fusion partner with Tpr in the transforming fusion oncogene *TPR-MET* in an immortalized osteosarcoma cell line that had been chemically mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine [Cooper *et al.* 1984]. In the Tpr-MET translocation the *TPR* gene (chromosome 1) is fused with the *MET* kinase gene (chromosome 7). Similar to other tyrosine kinase oncogenes, such as BCR-ABL and TEL-ABL, where the fusion partners BCR and TEL provide an oligomerization domain for the activation of ABL [Banerji and Sattler, 2004], the TPR sequence provides two leucine zipper domains, which facilitate oligomerization and substitute for ligand-stimulated activation. The structural changes as a result of the fusion with Tpr are thought to lead to the release of structural constraints that allow for constitutive activation of the HGFR kinase activity, thus turning it into a transforming protein [Zhen *et al.* 1994; Rodrigues and Park, 1993]. Other than in this example of chemically induced oncogenesis, there is little evidence that the TPR-MET translocation has clinical relevance or occurs *in vivo*.

HGFR expression has been described in a majority of normal cells and tissues. The receptor is synthesized as a single precursor that is posttranslationally digested and glycosylated, forming a 50 kDa extracellular α -chain and a transmembrane 140 kDa β -chain, which are then linked by disulfide bonds. HGFR is related to RON and the avian SEA receptor tyrosine kinases, which have extracellular structures related to the semaphorin receptor (or plexin) family

[Iwama *et al.* 1994; Huff *et al.* 1993; Ronsin *et al.* 1993]. The HGFR β -chain contains homologous structural domains shared with other proteins, including a semaphorin (sema) domain, a PSI domain (found within plexins, semaphorins, and integrins), four IPT repeats (found within immunoglobulins, plexins, and transcription factors), a transmembrane domain, a juxtamembrane (JM) domain, a tyrosine kinase domain, and a carboxy-terminal tail region.

HGFR is normally activated by ligation through its natural ligand HGF (or scatter factor). HGF is a member of the plasminogen-related growth factor family and was originally identified as a growth factor for hepatocytes and as a fibroblast-derived cell motility or scatter factor [Nakamura *et al.* 1987; Stoker *et al.* 1987; Gohda *et al.* 1986]. The HGF precursor is predominantly produced by mesenchymal cells, acting on epithelial cells [Sonnenberg *et al.* 1993; Stoker *et al.* 1987]. The secreted product is proteolytically cleaved into a disulfide-linked heterodimer. HGF consists of six domains (N-terminal domain, four kringle domains, and a C-terminal domain, which is structurally similar to the catalytic domain of serine proteinases). The stoichiometry of HGF binding to HGFR is 2:2. HGF has been shown to bind to the sema domain, suggesting an important role for activation and receptor dimerization [Gherardi *et al.* 2006; Stamos *et al.* 2004]. The HGFR sema domain contains seven beta sheets that fold into the shape of a seven-bladed propeller structure, where the second and third sheet bind to the HGF β -chain active site region [Stamos *et al.* 2004].

Aberrant HGFR activation can occur through HGF ligation or ligand-independent mechanisms. In transformed cells, the most likely mechanism of HGFR activation is overexpression and *MET* gene amplifications and mutations (germline and somatic) in a variety of malignancies. Thus, for the development of successfully targeted therapies, it will be necessary to understand the structural requirements involved in ligand-induced HGFR activation as well as the biochemical consequences that elicit a specific biological response. Advances in the development of targeted therapies for tyrosine kinase oncogenes suggest that HGFR may provide several domains that are rational targets for clinical therapeutics.

Dysregulation of HGFR expression

HGF-dependent autocrine HGFR activation has been found in human primary and metastatic tumors, including breast cancer, glioblastoma, osteosarcoma, melanoma, and others [Li *et al.* 2001; Koochekpour *et al.* 1997; Tuck *et al.* 1996; Ferracini *et al.* 1995]. Nonautocrine mechanisms can also activate the metastatic growth and potential of cancer cells. HGFR expressing tumor cells transplanted in HGF expressing transgenic mice resulted in pulmonary metastases, which could be altered by a HGF antagonist [Yu and Merlino, 2002]. The regulation of HGFR activity in oncogenic transformation is likely to be different compared with normal HGFR signaling. This is exemplified by the fact that overexpression of HGFR can have oncogenic potential by itself. Elevated HGFR levels and functional activation of the HGFR pathway have been shown to be sufficient for transformation of normal osteoblasts. *In vitro*, overexpression of HGFR resulted in the conversion of primary human osteoblasts into transformed osteosarcoma cells, also causing an osteosarcoma-like disease *in vivo* [Patane *et al.* 2006]. Overexpression of HGFR in hepatocytes is sufficient to induce hepatocellular carcinoma in transgenic mice [Wang *et al.* 2001]. Another important example is lung cancer, where HGFR overexpression is associated with higher pathological tumor stage and worse outcome [Rossi *et al.* 2005; Ichimura *et al.* 1996; Olivero *et al.* 1996]. HGFR was also found to be overexpressed in 20 of 52 squamous cell carcinomas, in 34 of 47 adenocarcinomas, and in all 11 non-small cell lung cancer (NSCLC) cell lines studied [Ichimura *et al.* 1996]. In addition to HGFR, higher levels of HGF can also be associated with a poorer prognosis in NSCLC [Siegfried *et al.* 1998]. In NSCLC, HGFR levels can be 2–10 times increased and HGF levels can be 10- to 100-fold higher compared with adjacent normal lung tissue [Siegfried *et al.* 1997]. Interestingly, susceptibility to carcinogen-induced lung cancer is increased in transgenic mice with HGF overexpression in the airways [Stabile *et al.* 2006]. A number of other proteins have been correlated with Met expression, and the downstream target paxillin and the transcription factor pax5 can be co-expressed with Met and/or phosphorylated Met in neuroendocrine tumors [Song *et al.* 2010]. Thus, in contrast to most cancers, at least in lung cancer, there does not seem to be a requirement for abnormal

expression of both HGF and its receptor for cancer susceptibility or development.

The transcriptional regulation of HGF is poorly understood, but several key factors that increase HGFR expression have been identified. For example, HGFR expression can be driven by hypoxia and hypoxia can increase HGF-dependent invasion [Pennacchietti *et al.* 2003]. In colorectal cancer, upregulation of HGFR may be an early event that is controlled by Wnt signaling [Boon *et al.* 2002]. Also, members of the Pax family of transcriptional activators involved in development are important regulators of HGFR expression. Both Pax3 and Pax7 modulate the expression of HGFR during limb muscle development, partially through unique mechanisms [Relaix *et al.* 2004; Epstein *et al.* 1996]. In lung cancer, PAX5 is expressed in SCLC and not NSCLC, whereas PAX8 is expressed in NSCLC and not SCLC [Kanteti *et al.* 2009]. PAX5 is shown to be a transcription factor for HGFR in SCLC and activated HGFR can be found within the nucleus. There are likely tissue- and lineage-specific additional factors that may act in concert with factors that function in a more general fashion to regulate HGFR expression. The participation of specific activators or co-activators of MET transcription in transformation of HGFR-dependent tumors is an intriguing possibility of additional therapeutic value that requires further attention.

Mutations of MET in cancer

There are two major mechanisms involving HGFR that can lead to activation or hyperresponsiveness of the HGFR/HGF pathway. MET can be either mutated in the extracellular or in the cytoplasmic domain (Table 2). Mutations can alter inhibitory constraints that allow the kinase to be either active or hyperresponsive to stimuli. Mutations can also alter the degree of activation or prevent efficient degradation of the protein, therefore prolonging the duration of the biochemical signals. Another mechanism of ligand-independent activation is the overexpression of the functional wild-type protein. Both mechanisms of HGFR activation have been observed, either individually or concomitantly. Tyrosine kinase mutations in *MET* were first identified in hereditary papillary renal cell carcinoma [Schmidt *et al.* 1997]. A systematic analysis of the complete exons, initially for lung cancer and thereafter for a number of other

Table 2. Hepatocyte growth factor receptor (HGFR) is a target of mutations in various malignancies. Known mutations within the semaphorin (SEMA), immunoglobulin plexin transcription (IPT), juxtamembrane (JM), and tyrosine kinase (TK) domain of MET are summarized. Mutations were found in non-small cell lung cancer (NSCLC), small-cell lung cancer (SCLC), mesothelioma, head and neck squamous cell carcinoma (HNSCC), melanoma, glioma, breast cancer, gastric cancer, and renal cell carcinoma in somatic (green) or germ line (orange) cells. There are two isoforms of HGFR and the amino acids are numbered according to the canonical isoform 1. The sequence of isoform 2 was used in some publications (differences indicated in brackets) and contains the following sequence after S755: TWWKEPLNIVSFLFCFAS [see also <http://www.uniprot.org> and [Krishnaswamy *et al.* 2009]]. Hepato, hepatocellular carcinoma. *Tissue not indicated in publication.

Mutation		NSCLC	SCLC	Mesothelioma	HNSCC	Melanoma	Hepato	Glioma	Breast	Gastric	Ovarian	Renal
SEMA	V136I											
	R143Q	Orange										
	S156L*											
	E168D	Green	Green								Green	
	L211W	Orange										
	L229F	Green	Green									
	L238S*											
	I316M*											
	A320V											Orange
	S323G	Green	Green									
	A347T	Orange										
	E355K	Orange										
	M362T	Orange										
	V370D	Green										
	N375S	Green	Green	Green		Green						
IPT	M431V			Green								
	N454I			Green								
JM	P773L									Orange		
	Del exon 10		Green									
JM	Del exon 14	Green										
	N930S (948)					Green						
	O960L (978)											Green
	G965V (983)					Green						
	R970C (988)	Green	Green			Green						
	R982S (1000)					Green						
	P991S (1009)									Green		
	T992I (1010)	Green	Green	Green					Green			
	S1040P (1058)	Green										
	G1085X			Green								
TK	V1092I (1110)											Green
	H1094R (1112)											Orange
	H1094L (1112)											Green
	H1094Y (1112)											Green
	H1106D (1124)											Green
	G1119V (1137)							Green				
	M1131T (1149)											Orange
	T1173I (1191)						Green					
	V1188L (1206)											Orange
	L1195V (1213)											Green
	V1220I (1238)											Orange
	D1228N (1246)											Orange
	D1228H (1246)											Green
	Y1230C (1248)				Green							Orange
	Y1230D (1248)											Orange
	Y1230H (1248)											Orange
	Y1235D				Green							Green
	K1244R (1262)						Green					
	M1250T (1268)											Green
	M1250I (1268)						Green					

solid tumors, revealed 'hot-spots' of mutations, especially in the sema and JM domains.

MET mutations in the semaphorin domain

The extracellular sema domain within MET is encoded by exon 2, and provides a specific binding site for HGF. Mutational analysis suggests that this domain is required for receptor activation and dimerization [Kong-Beltran *et al.* 2004]. The crystal structure of the HGFR sema domain revealed that it adopts a seven-blade β -propeller fold [Gherardi *et al.* 2006]. This important structural information will be helpful for the development of targeted therapies. Interference with the regulatory function of this domain would be expected to alter HGFR function [Kong-Beltran *et al.* 2004]. Interestingly, a portion of mesothelioma patients (7%) were found to have mutations (N375S, M431V, and N454I, the sequence of the canonical isoform 1 of HGFR was used for the numbering presented here) within this domain; however, the functional role of these MET mutations in transformation is not known [Jagadeeswaran *et al.* 2006]. Most recently, there have been germline MET mutations described in the sema domain in lung cancer [Krishnaswamy *et al.* 2009]. There is also a cluster of mutations that are different in various ethnic groups. As an example, N375S is identified in lung cancer patients from Asian and Caucasian origin; however, this mutation is not identified in African-American patients with lung cancer. In lung cancer, N375S is more common in squamous cell carcinomas and not adenocarcinomas or large-cell carcinomas. The squamous cell carcinomas harboring the N375S mutation were also smokers, whereas EGFR mutations are somatic, associated with adenocarcinomas, and more common in nonsmokers. These MET sema mutations result in differential binding to HGF, and the N375S mutation results in increased resistance to small-molecule inhibition.

MET mutations in the JM domain

Gain-of-function mutations in the JM domain have been well characterized for the receptor tyrosine kinase FLT3. They are present in about 20% of adult acute myeloid leukemia (AML) patients and thought to regulate the catalytic activity of the FLT3 kinase [Yokota *et al.* 1997]. Recently, a T992I JM domain MET mutation was identified in AML, although AML is usually not associated with JM domain mutations of MET [Loriaux *et al.* 2008]. The molecular mechanisms affected by JM mutations

in MET are not well characterized. Interestingly, MET mutations of the JM domain in Rottweiler dogs appear to increase cancer risk. The G966S gain-of-function germline mutation of MET occurs at a frequency of 74% in these dogs. Specifically, the carriers of this mutation appear to be predisposed to cancers, including lymphoma, osteosarcoma, and histiocytic sarcoma [Liao *et al.* 2006].

Some of the JM mutations found in human cancers may accelerate tumor formation rather than being fully transformed by themselves. A good example is the HGFR-T992I mutation, which is not associated with full activation of the HGFR kinase activity. However, tumors form faster in athymic nude mice injected with HGFR-T992I expressed in NIH3T3 cells compared with wild-type HGFR expressing cells [Lee *et al.* 2000].

In addition to the T992I mutation, R970C and T992A germline mutations were identified from 126 patients with adenocarcinomas. A screen of 79 mouse inbred strains also identified the R968C variation in SWR/J mice; genetic evidence suggests the importance of this variation in lung tumorigenesis [Zaffaroni *et al.* 2005]. The T992I mutation is present in some cases of hereditary papillary renal cell carcinoma and has been seen in a patient with breast cancer [Lee *et al.* 2000; Schmidt *et al.* 1999]. The same mutation is also found in the human H513 and H2596 mesothelioma cell lines [Jagadeeswaran *et al.* 2006]. Mutational analysis of SCLC tissue samples and cell lines revealed R970C, T992I, and S1040P mutations in the JM domain. The R970C mutation has been described in lung cancer from African-Americans and Caucasians but not in Asians. *In vitro* data suggest that these alterations may contribute to enhanced tumorigenicity, cell migration, and phosphorylation of HGFR protein in this disease [Ma *et al.* 2003]. Increased levels of reactive oxygen species (ROSs) have also been found to be associated with the R970C and T992I variants [Jagadeeswaran *et al.* 2007]. ROS in most cancer cells are of mitochondrial origin and do not only play a role in cancer cell signaling, but may also contribute to genomic instability [Rodrigues *et al.* 2008].

In 21% of human melanomas, the HGFR receptor was found to be phosphorylated at the Y1003 activation site. Further, a N930S HGFR

missense mutation was also identified in tumor tissue in addition to the HGFR-R970C mutation in melanoma cell lines [Puri *et al.* 2007]. The Y1003 residue is a binding site for the ubiquitin E3 ligase CBL and is crucial for the regulation of HGFR activity. When replaced by phenylalanine (Y1003F), a loss of ubiquitination of the HGFR receptor occurs and HGFR gains transforming activity in fibroblast and epithelial cells [Peschard *et al.* 2001]. The P991S germline mutation in the HGFR JM domain was found in a patient with gastric carcinoma. This mutation does not appear to induce ligand-independent activation of HGFR but it showed increased persistent response to HGF stimulation when expressed in fibroblasts [Lee *et al.* 2000].

MET mutations in the kinase domain

The kinase activity of HGFR is essential for the activation of signaling pathways and biological responses. Owing to its essential role, efforts had initially been directed on identifying mutations in the HGFR kinase domain of HGFR-expressing tumors. However, this mechanism of deregulating HGFR does not appear common and the majority of tyrosine kinase domain activating mutations in MET have been described in sporadic papillary renal carcinomas (somatic) and hereditary papillary renal cell carcinomas (germline) [Schmidt *et al.* 1997]. These mutations are sufficient to result in an increase in HGFR kinase activity and increased motility and metastasis [Jeffers *et al.* 1998; Schmidt *et al.* 1997]. Even though recent analyses aimed at identifying additional mutations have included all exons coding for the *MET* gene, it is conceivable that *in vivo*, the use of small-molecule tyrosine kinase inhibitors may lead to a reemergence of HGFR kinase domain mutations, as it has been shown for BCR-ABL and other kinases during targeted therapy [Walz and Sattler, 2006].

Amplification of MET

In addition to missense mutations, HGFR can also be activated by amplification. During development, the HGFR receptor is mainly expressed by epithelial cells but can also be found in a variety of human cancer cell lines or tumor tissue. Overexpression of MET occurs in many tumors to various degrees. For example, 10–20% of human gastric carcinomas have MET amplification [Sakakura *et al.* 1999], and gastric cancer cell lines show increased susceptibility to HGFR kinase inhibition [Smolen *et al.* 2006]. The breakage–fusion–bridge (BFB) mechanism

is thought to be a major cause for MET amplifications in these cancers [Hellman *et al.* 2002]. Interestingly, in NSCLC, amplification of MET strongly correlates with paxillin expression, a focal adhesion protein involved in the regulation of cytoskeletal functions. The potential prognostic value of paxillin expression in HGFR-dependent cancers is not known but it should be noted that paxillin is also a target of somatic mutations in approximately 9% of NSCLC [Jagadeeswaran *et al.* 2008]. Since amplification of MET is sufficient for transformation, one could envision MET amplification may also be a mechanism of a drug-resistant phenotype in cancers transformed by other oncogenes. *In vitro*, this mechanism has already been demonstrated in EGFR-dependent lung tumors that are resistant to the EGFR small-molecule inhibitor gefitinib [Engelman *et al.* 2007]. Although these data were not confirmed in the human disease [Bean *et al.* 2007], additional analysis of the mutational status of MET might provide a better understanding about its role in drug-resistant tumors.

HGF-dependent signaling through its receptor

Physiological activation of the HGFR signaling pathway is initiated by binding of soluble HGF to monomeric cell surface expressed mature HGFR receptors. HGF stimulation is followed by receptor dimerization, activation of its tyrosine kinase and rapid initiation of signaling cascades. One of the initial events of HGFR activation is thought to be phosphorylation at the three conserved tyrosine residues Y1230, Y1234, and Y1235 in the activation loop of the kinase domain. Phosphorylation at Y1234 and Y1235 correlates with increased tyrosine kinase activity [Rodrigues and Park, 1994] and is required for HGFR kinase activity [Longati *et al.* 1994]. Additional phosphorylation sites in HGFR lead to the recruitment of signaling proteins, which mediate downstream signaling events. These sites are either directly phosphorylated by HGFR or by other protein kinases. The unique multisubstrate docking sites Y1349 and Y1356 lead to the recruitment of a signaling complex when phosphorylated [Ponzetto *et al.* 1994]. Y1356 of HGFR is required for the binding of the adapter protein GRB2 through its SH2 (Src homology 2) domain [Nguyen *et al.* 1997]. Phosphorylated Y1349 interacts with the MBD (MET binding domain) containing adapter GAB1 (GRB2 associated binder 1), which may be sufficient for partial receptor interaction. The related GAB2 requires additional SH3 domain

binding from receptor bound GRB2 [Lock *et al.* 2002; Nguyen *et al.* 1997]. GAB1 is the major substrate for HGFR in epithelial cells and is also required for the morphogenic response [Nguyen *et al.* 1997; Weidner *et al.* 1996]. Cell morphogenesis is also mediated in part through Y1365 [Weidner *et al.* 1995]. An important regulator of this response with pleiotropic effects is phosphatidylinositol-3'kinase (PI3K), which is regulated indirectly through recruitment of Gab1 and binding of the p85 regulatory subunit of PI3K [Bardelli *et al.* 1997; Ponzetto *et al.* 1994]. Additional posttranslational modifications and domain structures are likely to contribute to the biological functions induced by the activated HGFR receptor and may also include nontyrosine residues that can alter HGFR function. As an example, S985 negatively regulates HGFR, likely involving phosphorylation by protein kinase C [Gandino *et al.* 1994].

A major difference between normal signaling through receptor activation and oncogenic, ligand-independent signaling is the transient nature of the stimulus. In transformed cells, regulatory mechanisms can be disrupted, whereas normal signaling is terminated by activation of specific phosphatases and internalization of HGFR into clathrin-coated vesicles. As part of the endosomal complex, HGFR is then finally degraded via the lysosomal pathway [Hammond *et al.* 2003, 2001]. Y1003 within the juxta-membrane domain of HGFR is an important regulatory site, which participates in this degradation process through recruitment of CBL, an E3-ubiquitin ligase. CBL binds to Y1003 when phosphorylated and facilitates ubiquitination of the HGFR receptor. HGFR containing the Y1003F mutation is not ubiquitinated and does not show altered HGFR internalization but increased stability of HGFR due to decreased lysosomal receptor degradation and thus further recycling to the membrane and signaling as well as oncogenic activation [Abella *et al.* 2005]. It would be predicted that the function of CBL is disrupted or altered at some level to enable prolonged or chronic signaling through HGFR in cancer cells. Activating mutations of CBL have been identified in AML and myeloproliferative disorders [Fernandes *et al.* 2010; Grand *et al.* 2009; Dunbar *et al.* 2008; Caligiuri *et al.* 2007; Sargin *et al.* 2007]. In lung cancer, novel CBL mutations may also indicate an essential role for tumorigenesis and metastasis [Tan *et al.* 2010]. Somatic CBL mutations were not found to be

mutually exclusive of MET or EGFR mutations, but they appear independent of p53 and KRAS mutations. In addition, pairwise analysis (normal/tumor) indicates a significant loss of heterozygosity (LOH) for the CBL locus (22%, $n=8/37$), in the absence of CBL mutations. CBL LOH was positively correlated with EGFR and MET mutations, suggesting an important role in EGFR and HGFR receptor regulation.

HGFR inhibition

Several novel agents targeting the HGFR pathway are currently under clinical investigation. (Table 3). Some of these molecules target other molecules along with HGFR. Ongoing clinical trials will potentially validate HGFR as a therapeutic target, improving the outcome of patients with HGFR-dependent cancers. HGFR small-molecule inhibitors have been utilized as single agents as well as in combination regimens. As we showed early on [Puri and Salgia, 2008], EGFR and HGFR synergize in biological functions such as cancer cell growth and cell motility/migration. There is also increased phosphorylation of various signal transduction molecules such as AKT and mammalian target of rapamycin (mTOR), thereby leading to activation. When combined with small-molecule inhibitors such as gefitinib, there was increased and synergistic apoptosis with a small-molecule MET inhibitor in a NSCLC cell line. Most recently, the small-molecule MET inhibitor SGX523 has been shown to be synergistic with erlotinib in preclinical models [Zhang *et al.* 2010]. Synergism has also been reported for MET inhibition with mTOR inhibition [Ma *et al.* 2005], PI3K inhibition [Ma *et al.* 2005], and cisplatin [Seiwert *et al.* 2009]. In addition, MET inhibition may have an additive effect when combined with topoisomerase-I inhibition in SCLC cells [Kanteti *et al.* 2009]. MET inhibition can also overcome sunitinib resistance, as well synergize with sunitinib [Shojaei *et al.* 2010].

In NSCLC, recent trials of two HGFR-targeted agents have suggested the efficacy of HGFR inhibition in combination with EGFR inhibition (utilizing erlotinib). A randomized controlled phase II trial of erlotinib combined with the HGFR selective TKI, ARQ 197 (also known as tivantinib), or placebo in patients with advanced NSCLC who had not been previously treated with EGFR inhibition did not demonstrate a significant improvement in progression-free survival (PFS) or overall survival (OS) in the intent-to-treat

Table 3. Hepatocyte growth factor (HGF) and HGF receptor inhibitors under clinical evaluation (see also <http://clinicaltrials.gov>).

Compound	Company	Mechanism of inhibition	Phase of study/type of tumor			
			I	II	III	IV
AMG102	Amgen	Human IgG2 monoclonal antibody against HGF that prevents receptor binding [Jun <i>et al.</i> 2007]				
			Active studies in multiple solid tumors			
AMG208	Amgen	MET kinase inhibitor				
			Active study in advanced solid tumors			
ARQ197	ArQule	MET kinase inhibitor [Garcia <i>et al.</i> 2007]				
			Completed studies in pancreatic adenocarcinoma. Active studies in colorectal cancer, hepatocellular carcinoma, NSCLC, refractory germ cell tumors			
BMS-777607	Bristol-Myers Squibb	MET kinase inhibitor [Schroeder <i>et al.</i> 2009]				
			Completed study in advanced solid tumors			
GSK1363089 (XL880)	GlaxoSmithKline	Tyrosine kinase inhibitor (MET, VEGFR2, PDGFR β , KIT, FLT3, TIE2, RON) [Bean <i>et al.</i> 2007]				
			Completed studies in Met ⁺ gastric cancer and squamous cell cancer of the head and neck. Active studies in papillary renal cell carcinoma, liver cancer, NSCLC, Met ⁺ breast cancer			
JNJ38877605	Johnson & Johnson	MET kinase inhibitor				
			Active study in advanced solid tumors			
MetMab	Genentech	Monovalent HGF antagonist antibody against MET [Jin <i>et al.</i> 2008; Martens <i>et al.</i> 2006]				
			Active study in NSCLC			
MGCD-265	MethylGene	MET, RON, TEK VEGFR1/2/3 kinase inhibitor				
			Active studies in advanced solid tumors and NSCLC			
MK2461	Merck	MET kinase inhibitor [Camacho <i>et al.</i> 2008]				
			Completed studies in advanced solid tumors			
MP470	SuperGen	Multi-targeted inhibitor of MET, RET, KIT, AXL, PDGFR α and FLT3 [Mahadevan <i>et al.</i> 2007]				
			Completed studies in advanced solid tumors			
PF-02341066	Pfizer	MET and ALK kinase inhibitor [Christensen <i>et al.</i> 2007; Zou <i>et al.</i> 2007]				
			Active studies in NSCLC, anaplastic large cell lymphoma, advanced solid tumors			
PF-04217903	Pfizer	MET kinase inhibitor [Christensen <i>et al.</i> 2007]				
			Suspended study in advanced solid tumors			
SGX523	SGX Pharm	MET kinase inhibitor	*	*early termination		
			Terminated studies in advanced solid tumors			
XL184	Exelixis	MET, VEGFR2, and RET kinase inhibitor				
			Active studies in glioblastoma, NSCLC, thyroid cancer, advanced solid tumors			

IgG, immunoglobulin; NSCLC, non-small cell lung cancer; PGDF(R), platelet-derived growth factor [receptor]; VEGF(R), vascular endothelial growth factor [receptor].

(ITT) population. However, there was a significant improvement in PFS in the ITT population when a Cox regression model was used to include key prognostic factors (hazard ratio [HR] 0.68, $p < 0.05$). In planned subset analyses, there was a significant improvement in both PFS and OS in patients with nonsquamous histology using the Cox regression model. In addition, patients with activating KRAS mutations gained significant improvement in PFS from treatment on the ARQ197-containing arm (HR 0.18) with a trend toward improvement in PFS in patients with wild-type EGFR as well (HR 0.7). The ARQ197-containing arm achieved an overall response rate of 10%, with additional responses after crossover from the placebo to ARQ197 containing arm [Schiller *et al.* 2010].

Currently, phase I and II studies are ongoing to evaluate the monovalent antagonist antibody to MET (MetMab; also known as onartuzumab). Preliminary phase I data demonstrated MetMab to be well tolerated in patients with advanced solid tumors, with a report of a complete response in a gastric cancer patient for a duration of approximately 2 years [Moss *et al.* 2010]. A randomized controlled phase II trial of MetMab in combination with erlotinib in patients with advanced NSCLC did not demonstrate an improvement in PFS or OS in the overall ITT population. However, the primary objective of this trial included the evaluation of PFS in patients with HGFR overexpression. Tumor tissue was obtained from 100% of the patients. A total of 54% patients had 'Met High' NSCLC by immunohistochemistry, as defined by 2+ or 3+ staining on a 0–3+ scale. In this predefined subset of patients, a trend towards improvement in both PFS (HR 0.56, $p = 0.0547$) and OS (HR 0.55, $p = 0.1113$) was seen. Interestingly, patients with low HGFR expression had a significantly worse PFS and OS with the addition of MetMab, compared with erlotinib alone. As a biomarker, high HGFR expression in the placebo arm was prognostic of a worse OS in patients treated with erlotinib alone (HR 2.52, $p = 0.0350$) [Spigel *et al.* 2010].

Conclusions

MET is involved in many mechanisms of cancer proliferation and metastasis. MET overexpression and genetic alterations play a role in the pathogenesis of several tumors, including lung cancer. The recent development of MET-targeted agents

offers the potential for improving patient outcomes in malignant diseases. The incorporation of further biomarker development studies of MET and other potential oncogenes into the design of clinical trials is essential to further individualize cancer care by carefully defining true prognostic and predictive markers in oncology.

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Conflict of interest statement

The authors declare no conflict of interest in preparing this document.

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