c-Met and Hepatocyte Growth Factor: Potential as Novel Targets in Cancer Therapy

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Current Oncology Reports 2007, **9:**102–108
Current Medicine Group LLC ISSN 1523-3790

Receptor tyrosine kinases have come to fruition as therapeutic targets in a variety of malignancies. In this group of targets, the c-Met receptor tyrosine kinase plays an important role in increased cell growth, reduced apoptosis, altered cytoskeletal function, increased metastasis, and other biologic changes. The ligand for c-Met is hepatocyte growth factor (HGF), also known as scatter factor. Met is overexpressed and mutated in a variety of malignancies, among which germline mutations are of particular interest. Most mutations of Met have been found in the juxtamembrane, the tyrosine kinase, and the semaphorin domain. Met gain-of-function mutations lead to deregulated or prolonged tyrosine kinase activity, which is instrumental to its transforming activity. This review summarizes the biologic functions regulated by Met and its structural requirements as well as related developments in targeted therapy. Treatment approaches, including antagonism of HGF binding to Met, targeting of RNA and the Met protein, and inhibition of the tyrosine kinase domain of Met, are highlighted. Targeting of the HGF/Met pathway, alone or in combination with standard therapies, is likely to improve current therapies in Met-dependent malignancies.

Introduction

c-Met is a receptor tyrosine kinase that has gained considerable interest through its deregulation or overexpression in a variety of cancers. It is activated and deregulated by its ligand hepatocyte growth factor (HGF), or through mutation or amplification. The activation can lead to a number of signal transduction cascades that then affect biologic and biochemical functions. Thus, Met may be an ideal rational target in clinical therapeutics.

The Met receptor tyrosine kinase was originally identified as the cellular homolog of the Tpr-Met oncoprotein [1]. The Tpr-Met translocation was created by treatment of the human osteogenic sarcoma cell line with the chemical carcinogen N-methyl-N'-nitro-N-nitrosoguanidine resulting in a fusion of the *Tpr* (chromosome 1) gene to the *Met* kinase gene (chromosome 7). The Tpr-Met oncoprotein has constitutively elevated Met tyrosine kinase activity, which is required for its transforming properties [2,3]. Met itself is mainly expressed on epithelial cells and is activated by its ligand HGF. HGF was originally identified as a growth factor for hepatocytes and as a fibroblast-derived cell motility factor or scatter factor [4–6]. HGF is a member of the plasminogen-related growth factor family. HGF precursor pro-HGF is cleaved by a protease to a disulfide-linked heterodimeric molecule, predominantly produced by mesenchymal cells. A number of biologic and biochemical functions for c-Met are summarized in this review, and novel approaches in the therapeutic inhibition of the HGF/Met pathway are detailed.

c-Met and Its Ligand HGF

The gene for human Met is located on chromosome 7 (7q21–q31) and encodes for a single precursor that is post-transcriptionally digested and glycosylated, forming a 50-kD extracellular α-chain and a transmembrane 140-kD β-chain, which are then linked by disulfide bonds. Met is related to Ron and Sea kinases, which have extracellular structures related to the semaphorin (sema) receptor (or plexin) family [7,8]. The Met β-chain contains structural homologous domains shared with other proteins, including a 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 (Fig. 1).

The ligand for c-Met has been identified as HGF. It was originally described as a potent mitogen for hepato-

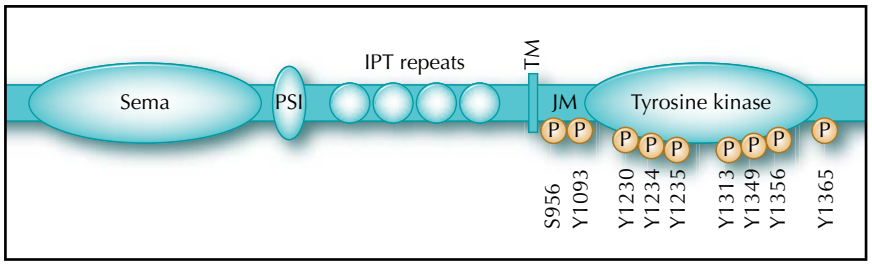

Figure 1. The functional domains of c-Met include the semaphorin-like (sema) domain, the PSI domain (found in plexins, semaphorins, and integrins), the IPT repeat domains (found in immunoglobulin-like regions, plexins, and transcription factors), the transmembrane (TM) domain, the juxtamembrane (JM) domain, the tyrosine kinase domain, and various phosphorylation sites (P) that are important for cellular functions.

cytes and later independently identified as scatter factor, a secretory protein of fibroblasts and smooth muscle cells [9]. HGF consists of six domains (N-terminal domain [n], four kringle domains [k1-k4], and a C-terminal domain [sp, structurally similar to the catalytic domain of serine proteinases]). There is a 2:2 stoichiometry of HGF binding to c-Met. HGF has been shown to bind to the sema domain [10,11]. The c-Met sema domain folds into a seven-blade β-propeller structure, where blades 2 and 3 bind to the HGF β-chain active site region.

c-Met Receptor Biology

Ligation of the Met receptor by its ligand HGF leads to receptor dimerization and activation of its intrinsic tyrosine kinase, followed by internalization into clathrin-coated vesicles, delivery to sorting endosomes, and degradation via the lysosomal pathway [12,13]. Phosphorylation of Met at Y1230, Y1234, and Y1235 in the activation loop of the tyrosine kinase domain correlates with increased tyrosine kinase activity [14,15]. Met activation can lead to autophosphorylation or phosphorylation of downstream intermediates and activation of signaling pathways. Also, Y1003 within the JM domain recruits c-Cbl when phosphorylated. c-Cbl is an E3-ubiquitin ligase that monoubiquinates the c-Met receptor, thereby directing internalization, trafficking to late endosomes, and ultimate degradation [16]. c-Cbl regulates internalization by acting as an adaptor for endophilin, an enzyme involved in membrane curvature [17,18]. Monoubiquitinated c-Met interacts with proteins of the endocytic pathway that contain ubiquitin-interacting domains, especially HGF-regulated tyrosine kinase substrate (Hrs). Hrs is believed to be involved in the retention of ubiquitinated receptors within the bilayered clathrin coat and in the recruitment of endosomal sorting complex required for transport complexes [19]. Hrs is tyrosine phosphorylated in response to HGF stimulation and required for internalization of c-Met.

Abella et al. [16] have shown that a ubiquitination-deficient c-Met (Y1003F, in the JM domain) reveals increased stability of c-Met (ie, decreased receptor degradation and thus further recycling to the membrane), signaling of downstream pathways, and oncogenic activation in vivo. Y1349 and Y1356 are unique multisubstrate docking sites that lead to the recruitment of a variety of proteins when phosphorylated, including SH2 (Src homology-2) domains, PTB (phosphotyrosine binding) domains, and MBD (Met binding domain) containing signaling proteins [20,21]. Phosphorylation of Y1313 in Met may not be required for complete functional activation of phosphatidylinositol 3-kinase (PI3K) through the receptor, even though this site is part of a putative Y-x-x-M binding motif for the recruitment of its regulatory p85 subunit [22]. The multisubstrate binding site of Met can recruit $p85^{PI3K}$, but the majority of PI3K activity is associated with the receptor indirectly through Gab1 [20,23]. Regulation of cell morphogenesis is mediated in part through Y1365 [24]. Met is also regulated through phosphorylation events independent of tyrosine residues (eg, the kinase activity of Met is negatively regulated through phosphorylation at S985) [25]. Additional interaction domains or phosphorylation sites in Met are likely to contribute to its functional regulation and stimulation of biologic activities.

Biologic Activities Regulated Through Activated c-Met

Signaling through the HGF/c-Met pathway has been demonstrated to trigger a variety of cellular responses that may vary based upon the cellular context. In vivo, HGF/c-Met signaling plays a role in growth, transformation of normal cells to malignant cells, cell motility, invasion, metastasis, epithelial to mesenchymal transition, angiogenesis, wound healing, and tissue regeneration. The regulation of Met activities in oncogenic transformation may be quite different from normal c-Met. HGF/Met has been implicated in embryonic development using mice with disruption of the *Met* gene [26–28]. Mice with *Met* gene disruption show embryonic lethality with severe defects in liver and placenta development, characteristics that are quite similar to those of mice with a disruption of the *HGF* gene. The HGF/Met pathway may have a broader role in morphogenesis and may regulate invasive growth and morphogenesis in multiple embryonic tissues, as has been suggested for the development of the nervous system [29].

Transformation

c-Met protooncogene was originally identified as the transforming fusion oncogene TPR-MET in an osteosarcoma cell line that had been chemically mutagenized in vitro [1]. The contribution of c-Met overexpression and activation in the transformation of normal cells has

recently been shown for osteoblasts [30]. Overexpression of c-Met by lentiviral vector-mediated gene transfer resulted in the conversion of primary human osteoblasts into osteosarcoma cells, displaying the transformed phenotype in vitro and the distinguishing features of human osteosarcoma in vivo. The transformation and tumorigenesis were fully abrogated when c-Met expression was inhibited by RNA interference or a dominant-negative form of c-Met. The role of c-Met and mutant c-Met in transforming normal human bronchial epithelial cells is not known. In small cell lung cancer (SCLC) and non–small cell lung cancer (NSCLC) cells, c-Met was found to be overexpressed [31,32]. c-Met receptor expression tended to be associated with higher pathologic tumor stage and worse outcome. Ichimura et al. [31] reported that Met was overexpressed in all 11 NSCLC cell lines studied, in 34 of 47 adenocarcinomas, and in 20 of 52 squamous cell carcinomas. Siegfried et al. [33] showed that the expression of Met was two- to 10-fold higher than in adjacent normal lung tissue in 25% of NSCLC tumors, and HGF levels were 10- to 100-fold higher in carcinoma samples compared with adjacent normal tissue. Higher levels of HGF were associated with more aggressive biology and a worse prognosis in NSCLC [34]. It was postulated that HGF, produced by mesenchymal cells within the tumor, acts upon epithelial cells that express its receptor, Met, thus representing a paracrine activation loop [35]. HGF overexpressing in the airways in transgenic mice increases susceptibility to carcinogenic-induced lung cancer [36]. In large cell neuroendocrine carcinoma of lung, c-Met was strongly expressed in 47% of tumor tissues and significantly correlated with survival in a univariate analysis [37].

Scattering

HGF was initially described as a secreted factor of fibroblasts that induced scattering, a process that involves active regulation of cytoskeletal functions and as a mitogen for hepatocytes [4–6]. The process of cell scattering can be divided into three phases: cell spreading, cell-cell dissociation, and cell migration. In order for epithelial cells to scatter, the disruption of cell-cell adhesions is required. Spontaneous cell scattering activity was shown in the c-Met expressing NCI-H358 lung adenocarcinoma cell line by retroviral gene transduction of HGF [38]. In addition, the HGF-overexpressing H358 cells show increased soft-agar colony formation and increased capacity to form xenograft tumors when implanted in the subcutaneous tissue of immune-deficient mice.

Cell motility

The formation and retraction of filopodia/lamellipodia, changes in actin formation, and cell migration are required for cell motility. HGF/c-Met signaling increases the motility of epithelial cells, and mutationally active Met induces the motility of Madin-Darby canine kidney cells [39]. In SCLC, cell motility is enhanced by HGF stimulation of c-Met RTK [40]. PI3K appears to be an important molecule in HGF-induced mito-, moto-, and morphogenesis, because inhibition of PI3K by wortmannin leads to decreased branching formation on a collagen matrix and chemotaxis of renal epithelial cells [41].

Invasion and metastasis

The mechanism by which HGF stimulation of c-Met leads to increased motility, migration, and invasion is not well understood. c-Met stimulation promotes cell movement, causes epithelial cells to disperse, and causes endothelial cells to migrate and promote chemotaxis [39]. Increased invasion is also likely to increase metastasis observed in solid tumors. Met-induced genes are likely to be actively involved in metastasis. Overexpression of c-Met in hepatocytes is sufficient to induce hepatocellular carcinoma in transgenic mice [42]. Similarly, Met-overexpressing tumor cells overexpressing HGF resulted in pulmonary metastasis in transgenic mice [43]. HGF-dependent autocrine Met activation has been found in human primary and metastatic tumors, including breast cancer, glioblastoma, osteosarcoma, and melanoma [44–47]. There may also be cross-talk between Met and other activated receptors for invasive growth signaling mechanisms. For example, activation of the lexin B1 tyrosine kinase related to Met has been shown to require Met expression for invasive growth. Ligation of plexin B1 leads to activation of Met, resulting in tyrosine phosphorylation of both receptors, and Met is required for invasive growth [48].

Met Is a Target of Mutations and Amplifications

The Met receptor is normally expressed by epithelial cells and has also been found to be expressed in a variety of human cancer cell lines or tumor tissue. Missense mutations of c-Met have been reported in a variety of cancers, with the majority of them identified in the cytoplasmic activation-loop tyrosine kinase domain [38]. Identification of activating mutations of c-Met in hereditary papillary renal carcinomas provided the first direct evidence linking c-Met directly to human oncogenesis. Germline missense mutations in the tyrosine kinase domain are detected in the majority of hereditary papillary renal cell carcinomas; somatic mutations have been found in some sporadic papillary renal carcinomas [49].

Besides missense mutations, c-Met–mediated tumorigenesis could be a result of gene amplification, as seen in human gastric carcinoma via the break-fusion-bridge mechanism [50]. Approximately 10% to 20% of gastric carcinomas can have c-Met amplification [51]. Smolen et al. [52] have shown that amplified c-Met gastric cancer cell lines are more susceptible to c-Met inhibition with PHA665752.

Mutations within the JM domain

JM domains of receptor tyrosine kinases have been identified as regulators of catalytic activity. For example, there are gain-of-function mutations in the receptor tyrosine kinase Flt3JM domain in approximately 20% of adult acute myeloid leukemias [53]. A germline mutation P1009S (exon 14) of c-Met was detected in a patient with gastric carcinoma and is the first such missense mutation to be described affecting the JM domain (as opposed to tyrosine kinase domain). The P1009S mutation does not induce ligand-independent activation of c-Met but shows increased persistent response to HGF stimulation when expressed in NIH3T3 cells [54]. Peschard et al. [55] have shown that the c-Cbl acts as a negative regulatory protein for c-Met, as well as several other RTKs, by promoting the polyubiquitination of c-Met. The Y1003 JM tyrosine, when replaced by phenylalanine (Y1003F), resulted in the loss of ubiquitination of the Met receptor and transformed activity in fibroblast and epithelial cells [55]. The JM domain T1010I mutation has been found in hereditary papillary renal cell carcinoma and in a patient with breast cancer [54,56]. This mutation may not lead to apparent activation of the Met receptor, but athymic nude mice injected with mutant Met in NIH3T3 cells form tumors slightly faster than wild-type Met-expressing cells [54]. In tissue samples and cell lines from SCLC, there are unique mutations in the JM domain (R988C, T1010I, and S1058P), which are likely to contribute to enhanced tumorigenecity, increased cell migration, and increased phosphorylation of protein in SCLC [57]. Furthermore, the R988C variation in c-Met was shown to be important in lung tumorigenesis of the SWR/J mouse strain (with Par4-susceptible allele) [58]. From 126 patients with adenocarcinomas in this study, it was shown that R988C and T1010I (and T1010A) were germline mutations. It is possible that these variations may affect lung cancer risk in carriers.

Tyrosine kinase–activating mutations

A unique activating Met mutation, fusing the *Tpr* and *Met* genes, led to the identification of Met itself [1]. The Tpr sequence provides two leucine zipper domains, which facilitate oligomerization and substitute for HGF-stimulated activation, resulting in constitutive activation of its kinase activity. The majority of activating mutations in Met have been described in sporadic papillary renal carcinomas and hereditary papillary renal cell carcinomas, resulting in an increase in kinase activity [39,49].

Semaphorin domain

The semaphorin-like (sema) domain is conserved among all semaphorins and is also found in the plexins and c-Met. In c-Met, the sema domain is encoded by exon 2 and binds specifically to HGF. More recently, the three-dimensional conformation of the HGF and heparin-binding sites of c-Met has been established by deletion mutagenesis of the RTK [59••]. The extracellular ligand-binding domain in the c-Met ectodomain was identified as adopting a seven-blade β-propeller fold for the sema domain of c-Met, homologous to the β-propeller fold template seen in the N-terminal domain of α_V-integrin. These three-dimensional models and functional map of the c-Met ectodomain would facilitate further development of targeted therapeutics against c-Met, in particular in cancers that overexpress Met. The unique role of the sema domain in the functional regulation of Met would also predict it to be a potential target of activating mutations in Met-dependent cancers.

Therapeutic Inhibition of Met Pathways

Unlike the small molecule kinase imatinib targeting BCR/ABL in chronic myelogenous leukemia and Kit in gastrointestinal stromal tumors, targeted small molecule inhibitors against c-Met have not come to clinical fruition yet. Deregulated activation of the Met pathway in transformed cells has led to the development of multiple approaches for targeted therapies, and a variety of Met pathway inhibitors have been identified that not only target Met itself but also disrupt the interaction of Met with its natural ligand HGF (Fig. 2). It is predicted that molecularly targeted therapy against Met will lead to dramatic inhibition of cancer growth and metastasis.

Small molecule Met kinase inhibitors

Several c-Met inhibitors are currently under evaluation [60–63], but their specificity may be limited, as it has been shown previously for imatinib mesylate [64]. Previous attempts have identified the broad-spectrum inhibitor K252a [62], which can also inhibit serine/threonine kinases. The novel small molecule Met inhibitor SU11274 (Pfizer, New York, NY) is effective in MET-transformed cell lines or lung cancer cells with activated Met as well but not in cells with activated ABL, JAK2, or PDGFβR kinases [63]. Inhibition of Met kinase activity reduced cell growth, induced G1 cell cycle arrest and apoptosis, and inhibited Met-dependent signaling. The identification of SU11274 as an effective inhibitor of Met tyrosine kinase activity illustrates the potential therapeutic use of targeting for Met in cancers associated with activated forms of this kinase. Additional specific Met inhibitors are under development, such as PHA-665752, a selective agent that inhibits Met-dependent effects in tumor cells and causes regression of GTL-16 gastric carcinoma xenografts [60].

Met peptide inhibitors

Peptides derived from the Met receptor have also been shown to inhibit Met signaling downstream of the activated receptor; however, clinical efficacy of these potential large molecule drugs may be limited. Peptides derived from the carboxy-terminal tail region bind Met and inhibit its kinase activity in vitro, including HGF-mediated invasion,

cell migration, and branched morphogenesis by almost 50% [65]. Inhibition of the Ron receptor tyrosine kinase by these peptides, likely due to homology, has also been identified [65].

Inhibition of HGF-mediated activation of Met

NK (N-terminal hairpin domain and kringle domain) inhibitors form a family of four variants of the HGF α-chain, containing one (NK1) to four (NK4) kringle domains. NK inhibitors act as weak agonists toward Met and antagonize binding of HGF to the receptor. Therefore, NK inhibitors would only be efficient against autocrine or paracrine activation of the Met receptor and likely not against activating mutations within Met. For example, angiogenesis as well as motility and invasion of HT115 human colorectal cancer cells has been reported to be antagonized by NK4 [66]. NK4 inhibited growth, invasion, and disseminated metastasis of pancreatic cancer cells in mice, resulting in prolonged lifespan [67]. Similarly, a decoy Met receptor was shown to interfere with extracellular signaling events in Met-dependent cancers by binding and therefore neutralizing HGF activity [68]. Another approach involves inhibition of HGF binding to c-Met with modified anti–c-Met antibodies. Targeting of Met-expressing glioblastoma in intracranial orthotopic xenografts has shown efficacy in vivo [69•].

Targeting Met expression

The expression of Met protein has been targeted at the RNA levels with Met-specific ribozymes and RNA interference (RNAi) or at the level of protein maturation. Small interference RNA (siRNA) is a novel approach, leading to the specific silencing of the targeted RNA. Ribozymes are RNA-based enzymes that bind to and cleave RNA molecules in a sequence-specific manner. Met-positive colorectal carcinoma has been targeted with ribozymes, reducing kinase activity of Met by up to 60% to 90% [70]. Whether ribozymes will sufficiently reduce Met levels in a clinical setting remains to be determined. Suppression of Met expression by RNAi is a promising novel approach, delivering siRNA to suppress the protein expression of Met. This approach has been successfully used in human cancer cell lines, including breast cancer, prostate cancer, sarcoma, glioblastoma, and gastric cancer cells. Reduction of Met expression led to reduced transformation, increased apoptosis, or inhibition of responsiveness to HGF stimulation [61,71]. Thus, with the advent of better means to deliver Met siRNA in vivo, this approach may be a suitable alternative to Met small molecule drug intervention in the treatment of Met-dependent cancers.

Met protein expression can be targeted at the level of protein maturation through inhibition of the heat shock protein (HSP90) by geldanamycin or related compounds of the anisomycin antibiotic family. HSP90 appears to be in an activated state in cancer cells, and HSP90 shows a high binding affinity for 17-allylaminogeldanamycin and

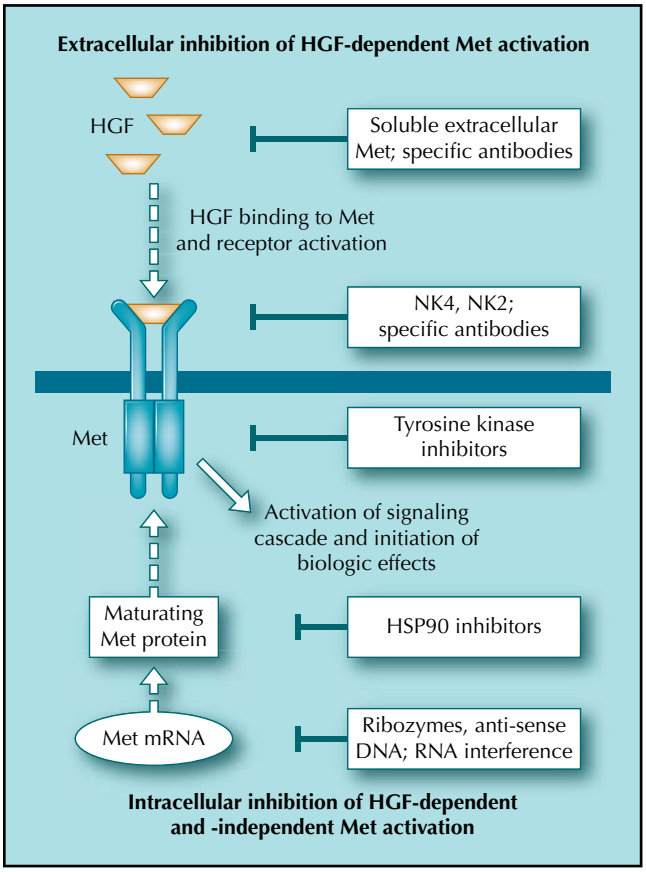

Figure 2. Targets for therapy within the hepatocyte growth factor (HGF)/Met pathway are shown. HGF-dependent activation of the Met pathway can be disrupted through extracellular therapies that interfere with HGF or extracellular Met. Intracellular approaches can attenuate or inhibit HGF-dependent and -independent mechanisms that lead to Met activation. HSP90—heat shock protein.

increased ATPase activity, which regulates HSP90 chaperone function [72]. Geldanamycins have been shown to inhibit maturation and functional expression of oncogenic proteins, including BCR-ABL and mutant p53, and are active against Met in SCLC cells as well, leading to reduced growth and viability [40]. Geldanamycins also block transformation of NIH3T3 cells expressing activating mutations or coexpressing HGF and Met [73]. A variety of proteins are thought to be downregulated by geldanamycins in addition to oncoproteins.

Conclusions

Emerging evidence indicates that the c-Met/HGF pathway is a quite powerful motor in driving tumor and normal cell biology. The overexpression, mutation, and amplification of c-Met in tumor cells underline its pleiotropic functions and hint at Met as an ideal target in clinical therapeutics. Results from several phase I clinical trials against c-Met and toxicity results are awaited. If the toxicity profile is reasonable, then efficacy phase II clinical trials against various tumors will be designed and implemented. As we

learn more about targeted therapies and combination with standard or other novel therapies, further clinical trials will likely be designed. Met is expected to be an important signaling target in a large number of malignancies.

Acknowledgments

M. Sattler is supported by the American Cancer Society and R. Salgia is supported in part by NIH/National Cancer Institute R01 grant, CA100750-03, American Lung Association, Institutional Cancer Research Awards from the University of Chicago Cancer Center with the American Cancer Society, and the V-Foundation.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Cooper CS, Park M, Blair DG, et al.: **Molecular cloning of a new transforming gene from a chemically transformed human cell line.** *Nature* 1984, **311:**29–33.
2. Rodrigues GA, Park M: **Dimerization mediated through a leucine zipper activates the oncogenic potential of the met receptor tyrosine kinase.** *Mol Cell Biol* 1993, **13:**6711–6722.
3. Zhen Z, Giordano S, Longati P, et al.: **Structural and functional domains critical for constitutive activation of the HGF-receptor (Met).** *Oncogene* 1994, **9:**1691–1697.
4. Gohda E, Tsubouchi H, Nakayama H, et al.: **Human hepatocyte growth factor in plasma from patients with fulminant hepatic failure.** *Exp Cell Res* 1986, **166:**139–150.
5. Nakamura T, Nawa K, Ichihara A, et al.: **Purification and subunit structure of hepatocyte growth factor from rat platelets.** *FEBS Lett* 1987, **224:**311–316.
6. Stoker M, Gherardi E, Perryman M, et al.: **Scatter factor is a fibroblast-derived modulator of epithelial cell mobility.** *Nature* 1987, **327:**239–242.
7. Comoglio PM, Boccaccio C: **The HGF receptor family: unconventional signal transducers for invasive cell growth.** *Genes Cells* 1996, **1:**347–354.
8. Maestrini E, Tamagnone L, Longati P, et al.: **A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor.** *Proc Natl Acad Sci U S A* 1996, **93:**674–678.
9. Stella MC, Comoglio PM: HGF: **a multifunctional growth factor controlling cell scattering.** *Int J Biochem Cell Biol* 1999, **31:**1357–1362.
10. Gherardi E, Sandin S, Petoukhov MV, et al.: **Structural basis of hepatocyte growth factor/scatter factor and MET signalling.** *Proc Natl Acad Sci U S A* 2006, **103:**4046–4051.
11. Stamos J, Lazarus RA, Yao X, et al.: **Crystal structure of the HGF beta-chain in complex with the Sema domain of the Met receptor.** *Embo J* 2004, **23:**2325–2335.
12. Hammond DE, Urbe S, Vande Woude GF, et al.: **Down-regulation of MET, the receptor for hepatocyte growth factor.** *Oncogene* 2001, **20:**2761–2770.
13. Teis D, Huber LA: **The odd couple: signal transduction and endocytosis.** *Cell Mol Life Sci* 2003, **60:**2020–2033.
14. Kamada M, Komori A, Chiba S, et al.: **A prospective study of congenital cytomegalovirus infection in Japan.** *Scand J Infect Dis* 1983, **15:**227–232.
15. Rodrigues GA, Park M: **Autophosphorylation modulates the kinase activity and oncogenic potential of the Met receptor tyrosine kinase.** *Oncogene* 1994, **9:**2019–2027.
16. Abella JV, Peschard P, Naujokas MA, et al.: **Met/Hepatocyte growth factor receptor ubiquitination suppresses transformation and is required for Hrs phosphorylation.** *Mol Cell Biol* 2005, **25:**9632–9645.
17. Petrelli A, Gilestro GF, Lanzardo S, et al.: **The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met.** *Nature* 2002, **416:**187–190.
18. Soubeyran P, Kowanetz K, Szymkiewicz I, et al.: **Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors.** *Nature* 2002, **416:**183–187.
19. Bache KG, Raiborg C, Mehlum A, et al.: **STAM and Hrs are subunits of a multivalent ubiquitin-binding complex on early endosomes.** *J Biol Chem* 2003, **278:**12513–12521.
20. Ponzetto C, Bardelli A, Zhen Z, et al.: **A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family.** *Cell* 1994, **77:**261–271.
21. Furge KA, Zhang YW, Vande Woude GF: **Met receptor tyrosine kinase: enhanced signaling through adapter proteins.** *Oncogene* 2000, **19:**5582–5589.
22. Fournier TM, Kamikura D, Teng K, et al.: **Branching tubulogenesis but not scatter of madin-darby canine kidney cells requires a functional Grb2 binding site in the Met receptor tyrosine kinase.** *J Biol Chem* 1996, **271:**22211–22217.
23. Bardelli A, Longati P, Gramaglia D, et al.: **Gab1 coupling to the HGF/Met receptor multifunctional docking site requires binding of Grb2 and correlates with the transforming potential.** *Oncogene* 1997, **15:**3103–3111.
24. Weidner KM, Sachs M, Riethmacher D, et al.: **Mutation of juxtamembrane tyrosine residue 1001 suppresses loss-of-function mutations of the met receptor in epithelial cells.** *Proc Natl Acad Sci U S A* 1995, **92:**2597–2601.
25. Gandino L, Longati P, Medico E, et al.: **Phosphorylation of serine 985 negatively regulates the hepatocyte growth factor receptor kinase.** *J Biol Chem* 1994, **269:**1815–1820.
26. Bladt F, Riethmacher D, Isenmann S, et al.: **Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud.** *Nature* 1995, **376:**768–771.
27. Schmidt C, Bladt F, Goedecke S, et al.: **Scatter factor/hepatocyte growth factor is essential for liver development.** *Nature* 1995, **373:**699–702.
28. Uehara Y, Minowa O, Mori C, et al.: **Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor.** *Nature* 1995, **373:**702–705.
29. Maina F, Klein R: **Hepatocyte growth factor, a versatile signal for developing neurons.** *Nat Neurosci* 1999, **2:**213–217.
30. Patane S, Avnet S, Coltella N, et al.: **MET overexpression turns human primary osteoblasts into osteosarcomas.** *Cancer Res* 2006, **66:**4750–4757.
31. Ichimura E, Maeshima A, Nakajima T, et al.: **Expression of c-met/HGF receptor in human non-small cell lung carcinomas in vitro and in vivo and its prognostic significance.** *Jpn J Cancer Res* 1996, **87:**1063–1069.
32. Olivero M, Rizzo M, Madeddu R, et al.: **Overexpression and activation of hepatocyte growth factor/scatter factor in human non-small-cell lung carcinomas.** *Br J Cancer* 1996, **74:**1862–1868.
33. Siegfried JM, Weissfeld LA, Singh-Kaw P, et al.: **Association of immunoreactive hepatocyte growth factor with poor survival in resectable non-small cell lung cancer.** *Cancer Res* 1997, **57:**433–439.
34. Siegfried JM, Weissfeld LA, Luketich JD, et al.: **The clinical significance of hepatocyte growth factor for non-small cell lung cancer.** *Ann Thorac Surg* 1998, **66:**1915–1918.
35. Tokunou M, Niki T, Eguchi K, et al.: **c-MET expression in myofibroblasts: role in autocrine activation and prognostic significance in lung adenocarcinoma.** *Am J Pathol* 2001, **158:**1451–1463.
36. Stabile LP, Lyker JS, Land SR, et al.: **Transgenic mice overexpressing hepatocyte growth factor in the airways show increased susceptibility to lung cancer.** *Carcinogenesis* 2006, **27:**1547–1555.

37. Rossi G, Cavazza A, Marchioni A, et al.: **Role of chemotherapy and the receptor tyrosine kinases KIT, PDGFRalpha, PDGFRbeta, and Met in large-cell neuroendocrine carcinoma of the lung.** *J Clin Oncol* 2005, **23:**8774–8785.
38. Yi S, Tsao MS: **Activation of hepatocyte growth factor-met autocrine loop enhances tumorigenicity in a human lung adenocarcinoma cell line.** *Neoplasia* 2000, **2:**226–234.
39. Jeffers M, Fiscella M, Webb CP, et al.: **The mutationally activated Met receptor mediates motility and metastasis.** *Proc Natl Acad Sci U S A* 1998, **95:**14417–14422.
40. Maulik G, Kijima T, Ma PC, et al.: **Modulation of the c-Met/hepatocyte growth factor pathway in small cell lung cancer.** *Clin Cancer Res* 2002, **8:**620-627.
41. Derman MP, Chen JY, Spokes KC, et al.: **An 11-amino acid sequence from c-met initiates epithelial chemotaxis via phosphatidylinositol 3-kinase and phospholipase C.** *J Biol Chem* 1996, **271:**4251-4255.
42. Wang R, Ferrell LD, Faouzi S, et al.: **Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice.** *J Cell Biol* 2001, **153:**1023-1034.
43. Yu Y, Merlino G: **Constitutive c-Met signaling through a nonautocrine mechanism promotes metastasis in a transgenic transplantation model.** *Cancer Res* 2002, **62:**2951-2956.
44. Tuck AB, Park M, Sterns EE, et al.: **Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma.** *Am J Pathol* 1996, **148:**225–232.
45. Koochekpour S, Jeffers M, Rulong S, et al.: **Met and hepatocyte growth factor/scatter factor expression in human gliomas.** *Cancer Res* 1997, **57:**5391–5398.
46. Li G, Schaider H, Satyamoorthy K, et al.: **Downregulation of E-cadherin and Desmoglein 1 by autocrine hepatocyte growth factor during melanoma development.** *Oncogene* 2001, **20:**8125–8135.
47. Ferracini R, Di Renzo MF, Scotlandi K, et al.: **The Met/HGF receptor is over-expressed in human osteosarcomas and is activated by either a paracrine or an autocrine circuit.** *Oncogene* 1995, **10:**739–749.
48. Giordano S, Corso S, Conrotto P, et al.: **The semaphorin 4D receptor controls invasive growth by coupling with Met.** *Nat Cell Biol* 2002, **4:**720–724.
49. Schmidt L, Duh FM, Chen F, et al.: **Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas.** *Nat Genet* 1997, **16:**68–73.
50. Hellman A, Zlotorynski E, Scherer SW, et al.: **A role for common fragile site induction in amplification of human oncogenes.** *Cancer Cell* 2002, **1:**89–97.
51. Sakakura C, Mori T, Sakabe T, et al.: **Gains, losses, and amplifications of genomic materials in primary gastric cancers analyzed by comparative genomic hybridization.** *Genes Chromosomes Cancer* 1999, **24:**299–305.
52. Smolen GA, Sordella R, Muir B, et al.: **Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752.** *Proc Natl Acad Sci U S A* 2006, **103:**2316–2321.
53. Yokota S, Kiyoi H, Nakao M, et al.: **Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines.** *Leukemia* 1997, **11:**1605–1609.
54. Lee JH, Han SU, Cho H, et al.: **A novel germ line juxtamembrane Met mutation in human gastric cancer.** *Oncogene* 2000, **19:**4947–4953.
55. Peschard P, Fournier TM, Lamorte L, et al.: **Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein.** *Mol Cell* 2001, **8:**995–1004.
56. Schmidt L, Junker K, Nakaigawa N, et al.: **Novel mutations of the MET proto-oncogene in papillary renal carcinomas.** *Oncogene* 1999, **18:**2343–2350.
57. Ma PC, Kijima T, Maulik G, et al.: **c-MET mutaional anlysis in small cell lung cancer: Novel juxtamembrance domain mutations regulating cytoskeletal functions.** *Cancer Res* 2003, **63:** 6272–6281.
58. Zaffaroni D, Spinola M, Galvan A, et al.: **Met proto-oncogene juxtamembrane rare variations in mouse and humans: differential effects of Arg and Cys alleles on mouse lung tumorigenesis.** *Oncogene* 2005, **24:**1084–1090.
59.•• Kong-Beltran M, Stamos J, Wickramasinghe D: **The Sema domain of Met is necessary for receptor dimerization and activation.** *Cancer Cell* 2004, **6:**75–84.

This study characterizes the sema domain in Met as an important regulator of receptor activation and dimerization.

60. Christensen JK, Schreck R, Burrows J, et al.: **A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive anti-tumor activity in vivo.** *Cancer Res* 2003, **63:**7345–7355.
61. Cassinelli G, Lanzi C, Petrangolini G, et al.: **Inhibition of c-Met and prevention of spontaneous metastatic spreading by the 2-indolinone RPI-1.** *Mol Cancer Ther* 2006, **5:**2388–2397.
62. Morotti A, Mila S, Accornero P, et al.: **K252a inhibits the oncogenic properties of Met, the HGF receptor.** *Oncogene* 2002, **21:**4885–4893.
63. Sattler M, Pride YB, Ma P, et al.: **A novel small molecule met inhibitor induces apoptosis in cells transformed by the oncogenic TPR-MET tyrosine kinase.** *Cancer Res* 2003, **63:**5462–5469.
64. Walz C, Sattler M: **Novel targeted therapies to overcome imatinib mesylate resistance in chronic myeloid leukemia (CML).** *Crit Rev Oncol Hematol* 2006, **57:**145–164.
65. Bardelli A, Longati P, Williams TA, et al.: **A peptide representing the carboxyl-terminal tail of the met receptor inhibits kinase activity and invasive growth.** *J Biol Chem* 1999, **274:**29274–29281.
66. Parr C, Hiscox S, Nakamura T, et al.: **Nk4, a new HGF/SF variant, is an antagonist to the influence of HGF/SF on the motility and invasion of colon cancer cells.** *Int J Cancer* 2000, **85:**563–570.
67. Tomioka D, Maehara N, Kuba K, et al.: **Inhibition of growth, invasion, and metastasis of human pancreatic carcinoma cells by NK4 in an orthotopic mouse model.** *Cancer Res* 2001, **61:**7518–7524.
68. Michieli P, Basilico C, Pennacchietti S, et al.: **Mutant Met-mediated transformation is ligand-dependent and can be inhibited by HGF antagonists.** *Oncogene* 1999, **18:**5221–5231.
69.• Martens T, Schmidt NO, Eckerich C, et al.: **A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo.** *Clin Cancer Res* 2006, **12:**6144–6152.

Interesting study demonstrating the activity of a modified anti-Met antibody in Met-dependent tumorigenesis.

70. Herynk MH, Stoeltzing O, Reinmuth N, et al.: **Down-regulation of c-Met inhibits growth in the liver of human colorectal carcinoma cells.** *Cancer Res* 2003, **63:**2990–2996.
71. Shinomiya N, Gao CF, Xie Q, et al.: **RNA interference reveals that ligand-independent met activity is required for tumor cell signaling and survival.** *Cancer Res* 2004, **64:**7962–7970.
72. Kamal A, Thao L, Sensintaffar J, et al.: **A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors.** *Nature* 2003, **425:**407–410.
73. Webb CP, Hose CD, Koochekpour S, et al.: **The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network.** *Cancer Res* 2000, **60:**342–349.