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Review Article

HGF- and c-Met-targeted drugs: hopes, challenges and their future in cancer therapy

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Abstract

Cancer remains a formidable disease, with 1,372,910 new cases and 570,280 deaths in the United States alone in 2005 (http://www.cancer.org/docroot/stt/stt_0.asp). Many types of cancers are still difficult to treat using conventional therapies. For example, chemotherapeutic drugs are widely used, but their side effects can be devastating to the quality of life of patients and their efficacy is limited in late-stage cancers. Therefore, efforts have been undertaken for the past several years to develop targeted drugs in the hope of improving efficacy and tolerability. As these new drugs are starting to make their mark in the clinic, what can we expect in the future with anticancer drugs? Already we are seeing the benefits of targeted therapies, different drug modalities, including antibodies, and more convenient forms of drug delivery. Moreover, much attention is now being placed not only on attacking the cancer cells themselves, but also on intervening in the microenvironment in which they reside. In this review, we will discuss the promise behind novel targeted therapies under development to inhibit signaling via the oncogenic HGF/c-Met pathway. HGF and c-Met are exciting therapeutic targets that play important roles both directly in tumor cells and in the tumor microenvironment.

Introduction

c-Met is a receptor tyrosine kinase (RTK) originally identified as an oncogenic fusion protein (TPR-MET) with constitutive activity (1). Soon after the identification of the receptor, hepatocyte growth factor/scatter factor (HGF/SF, hereafter referred to as HGF) was isolated, cloned, characterized (2-4) and subsequently recognized as the ligand for c-Met (5). In response to HGF binding, the c-Met kinase domain becomes autophosphorylated. This in turn triggers the phosphorylation of tyrosine residues at the *C*-terminal tail of the receptor that constitute a unique docking site for interaction with adaptor proteins essential for initiation of signaling cascades (reviewed in 6).

HGF/c-Met signaling mediates a diverse array of biological activities, including proliferation, survival, motility, migration, branching morphogenesis, wound healing and angiogenesis (6). These activities are essential during embryogenesis and tissue repair, but they also contribute to cancer progression by enabling tumor cell proliferation, survival, invasion, metastasis and angiogenesis (6-8). HGF and c-Met have generated significant interest in the oncology research community because: 1) evidence that their dysregulation leads to oncogenesis is compelling and 2) both the ligand and the receptor are amenable to therapeutic intervention by multiple modalities. These two characteristics make the HGF/c-Met axis ideal for the development of targeted therapies.

Roles of HGF and c-Met in tumorigenesis

Clinically, dysregulation of the HGF/c-Met axis has been observed in numerous types of cancer (6) (http://www.vai.org/vari/metandcancer/index.aspx). In normal tissue, HGF is produced by stromal cells and c-Met is expressed on epithelial cells. Thus, HGF typically acts on its receptor in a paracrine manner. In cancerous tissues, aberrant activation of c-Met can occur in a variety of ways. HGF and/or c-Met can be overexpressed and/or amplified while maintaining a paracrine loop. Alternatively, HGF can be misexpressed by the epithelial cells, thereby establishing an autocrine loop in which the tumor cells express both ligand and receptor. Ligand-independent activation of c-Met has also been observed. This can occur in cancer cells that overexpress c-Met or cells that carry an activating c-Met mutation (6, 7). These

different scenarios have been modeled *in vitro* and in animals to evaluate their contribution to tumorigenesis. This section concentrates on animal models, as the *in vitro* activities of HGF and c-Met have been reviewed recently (6, 9).

Several mouse models overexpressing HGF have demonstrated a role for this growth factor in tumorigenesis. Transgenic mice expressing wild-type murine HGF under the metallothionein (MT) promoter developed a variety of tumor types of both mesenchymal and epithelial origin between 2.5 and 20 months of age (10, 11). Coexpression of HGF and c-Met in many of these tumors suggests an autocrine loop as the mechanism of transformation (11). When mouse HGF was targeted to the mammary epithelium under the whey acidic protein (WAP) promoter, a large percentage of mice developed mammary tumors with a latency of approximately 10 months. Lung metastases were found in 22% of transgenic mice, supporting a role for HGF in metastatic growth (12). Expression of HGF in the mammary epithelium again established an autocrine loop for HGF/c-Met signaling, providing further support that the creation of an autocrine loop can contribute to tumorigenesis. Another group showed that expression of HGF under the albumin (ALB) promoter resulted in grossly visible liver tumors with a long latency of 17 months (13). Thus, HGF overexpression induces tumorigenesis, usually with a long latency, suggesting that secondary hits may be necessary. This conclusion is strengthened by studies using the carcinogen diethylnitrosamine (DEN) as an initiator in MT-HGF or ALB-HGF mice. Under these conditions, tumor development in the liver was accelerated (13, 14).

New tools were recently developed to enable examination of the paracrine action of HGF in xenograft models (15, 16). In one instance, SCID mice were generated expressing human HGF under the MT promoter (hHGF-Tg SCID) (15). Xenograft tumors that expressed c-Met and responded to HGF *in vitro* grew at an enhanced rate in hHGF-Tg mice compared to control mice. The growth of xenografts that express minimal levels of c-Met was not affected. In the second model, human HGF was expressed in mouse lung via the Clara cell secretory protein (CCSP) promoter (16). The HGF transgenic mice developed lung tumors more frequently than control animals after exposure to a tobacco carcinogen. Moreover, HGF levels in the tumors of the HGF transgenic mice were higher than in the tumors of the controls. Taken together, the HGF transgenic models provide evidence that overexpression of HGF in a paracrine manner or creation of an autocrine loop can contribute to tumorigenesis and metastasis, and suggest the potential utility of HGF inhibitors as targeted antitumor agents.

Transgenic c-Met models demonstrating tumorigenicity have also been developed. Interestingly, overexpression of wild-type c-Met is not always sufficient for tumor development. For example, overexpression of wild-type c-Met in primary mammary epithelial cells transplanted into the mammary fat pad led to the development of nonprogressive neoplasms (17). On the other hand, co-expression of wild-type c-Met with a second proto-oncogene, c-myc, led to the development of palpable tumors within 10 weeks (17). In another model, c-Met overexpression led to tumor growth, but latency was long and penetrance was incomplete (18). However, upon repression of the c-Met transgene, tumors regressed, supporting a maintenance role for c-Met in tumors. Moreover, this model demonstrates the possibility that ligand-independent activation of c-Met can lead to oncogenesis (18). Indeed, the only source of HGF in this study is of mouse origin and mouse HGF is unable to activate human c-Met used in this study (19).

While wild-type c-Met is a weak oncogene in the absence of HGF, activated versions of c-Met readily form tumors in mice. Transgenic mice expressing the oncogenic fusion protein TPR-MET under the control of the MT promoter developed mammary tumors within 6 months (20). TPR-MET was originally identified in a human cell line as a result of *in vitro* mutagenesis that generated a chromosome translocation. Although there are conflicting reports as to the importance of this translocation and fusion protein in spontaneous human tumors (21-23), this model is useful in that it demonstrates that constitutive activation of c-Met is oncogenic. This has been confirmed with spontaneously occurring mutations of c-Met. Indeed, c-Met mutations identified from cancer patients are oncogenic when transfected into NIH/3T3 cells. Cells transfected with a number of the c-Met mutants showed high basal kinase activity and were phenotypically transformed, whereas cells expressing wild-type c-Met were phenotypically normal. Moreover, cells expressing the mutant c-Met alleles formed tumors more readily in nude mice than cells expressing wild-type c-Met. Cells expressing one of the activated c-Met mutants were also highly metastatic in an *in vitro* assay (24-26). Interestingly, cell transformation with some c-Met mutants was HGF-dependent. In fact, transformation by these mutants could be inhibited by HGF antagonists (27). These data suggest that tumors carrying c-Met TK-activating mutations may potentially be responsive to HGF antagonists. Moreover, such tumors should also respond well to c-Met inhibitors.

Attacking the HGF/c-Met axis from several angles

Early attempts to create a protein therapeutic to intervene in the HGF/c-Met signaling axis used truncated forms of the HGF protein. The best-characterized molecule in this class of antagonists is the NK4 molecule containing the *N*-terminal 447 amino acids of the α-chain, which includes the hairpin and the 4 kringle domains of the full-length protein (28). NK4 was shown to be an antagonist due to its ability to bind c-Met without activating it, thereby competitively blocking the interaction of active HGF with c-Met. This molecule has shown efficacy in multiple mouse models, including orthotopic glioblastoma and pancreatic tumors (29, 30). However, because NK4 binds the c-Met receptor with 10-fold lower affinity compared to native HGF (28), NK4 may be somewhat

limited as a suitable molecule for inhibition of the HGF/c-Met axis. Interestingly, NK4 also has antiangiogenic activities independent of c-Met and is able to block basic fibroblast growth factor (bFGF)- and vascular endothelial growth factor (VEGF)-induced angiogenesis in a rabbit corneal assay (reviewed in 31). The mechanism by which NK4 exerts this antiangiogenic activity, although not well understood, is thought to be due to its similarity to angiostatins. However, this trait may lend additional clinical utility to a molecule that may not be able to achieve full inhibition of the HGF/c-Met axis in patients.

Ribozymes have also been explored for their ability to block the HGF/c-Met axis. Ribozymes are small nucleic acid constructs with enzymatic activity that specifically cleave their targeted mRNA. Hammerhead ribozymes that target c-Met and/or HGF have proven to be effective in downregulating their target proteins in multiple cell lines, including the U-87 MG glioblastoma line (32) and the PC-3-LN4 subclone of a prostate cancer line (33). In mouse models, systemic downregulation can be achieved by using a lentivirus delivery system, resulting in reduced tumor take, size and, in the case of the PC-3 model, metastasis. However, translation of this approach to the clinic may be difficult due to the current challenges that exist related to gene therapy.

Soluble and dominant negative forms of c-Met have also been proposed as potential therapeutics. Studies suggest that the soluble decoy receptors are more effective than NK4 at blocking HGF-induced c-Met autophosphorylation, mitogenesis and c-Met-dependent wound healing (34). One study demonstrated that a soluble form of the sema domain of the c-Met receptor alone can effectively block c-Met signaling *in vitro* (35). An alternative delivery system using a lentivirus was used to express the soluble c-Met extracellular domain in cells *in vitro* or systemically in animals (34). This approach was demonstrated to inhibit tumor growth and metastasis in an MDA-MB-435-b4 breast cancer xenograft model. Like the ribozyme, however, delivery is via a gene therapy system and would face considerable clinical hurdles, although soluble proteins may be a more viable approach.

Neutralizing antibodies are another protein-based approach to intervene clinically in this signaling axis. Multiple groups have reported on the success of using monoclonal antibodies to neutralize HGF/c-Met signaling *in vitro* and in mouse models. Attempts to generate antibodies against the c-Met receptor have focused on the sema domain of c-Met (35), which can bind to the β-chain of HGF and is responsible for dimerization of the receptor (36). Single-chain antibodies generated to this region of c-Met are effective at blocking HGF-triggered autophosphorylation in multiple cell types, as well as being capable of functionally blocking cell motility and migration (35). However, *in vivo* data have not been reported using this antibody.

Neutralizing monoclonal antibodies against HGF have also been reported. The first report showed that a combination of three monoclonal antibodies was necessary to inhibit scattering and block the growth of U-118 glioblastoma tumor xenografts (37). While interesting as a proof-of-principle study, the approach of using pooled monoclonal antibodies is not practical from a clinical standpoint due to the difficulty in developing and delivering three different agents as a single therapeutic.

More recent reports have demonstrated that single neutralizing antibodies to HGF can effectively block HGF signaling. Both a mouse monoclonal, known as L2G7 (38), and fully human monoclonal antibodies, known as 2.12.1 and 2.4.4 (39), have been reported to bind HGF, neutralize the interaction between HGF and c-Met, and functionally inhibit U-87 MG cells *in vitro* and U-87 MG tumor xenografts *in vivo*. U-87 MG is a human glioblastoma-derived cell line whose growth is driven by HGF signaling. In the case of L2G7, the antibody demonstrated efficacy against a U-87 MG orthotopic model when administered systemically, providing evidence that it can cross the blood-brain/tumor barrier and suggesting clinical application for anti-HGF antibodies in glioblastoma multiforme (GBM) patients. Importantly, 2.12.1 demonstrates additive antitumor effects in combination with temozolomide, a drug currently used for GBM patients, in the U-87 MG model, further supporting the clinical potential of anti-HGF therapy in GBM (T. Jun, manuscript in preparation). Clinical trials are ongoing with an antibody against HGF (http://www-ext.amgen.com/investors/pipe_AMG102.html).

The antibody and antagonist protein approaches have the advantage of being exquisitely specific for their targets. However, antibodies against HGF in theory will benefit only those patients who have HGF-dependent tumors. Small-molecule kinase inhibitors may provide an opportunity to combat ligand-independent disease without resorting to gene therapy approaches. Data have recently emerged describing a few small molecules that specifically target c-Met.

SU-11274 is an ATP-competitive inhibitor of c-Met. This molecule inhibits c-Met phosphorylation in HGF-stimulated cells and inhibits functional activities mediated by c-Met, such as cell scatter and invasion (40, 41). Interestingly, this molecule can inhibit the constitutive c-Met activity of some c-Met mutants, although other mutants are resistant to the inhibitory activity of SU-11274 (42). The homology model used to predict the structure of c-Met could not explain the mechanism of resistance based on the location of the specific amino acids. While Y1248H is located in the activation loop of the kinase domain, L1213V is located in the hinge region, far from any point of contact between SU-11274 and c-Met. It is possible that the L1213V substitution leads to a conformational change in the protein that alters the affinity of SU-11274 for c-Met. It would be interesting to determine the crystal structure of these c-Met mutants to elucidate their mechanism of resistance to molecules such as SU-11274. This information would also be useful to design molecules that can inhibit L1213V and Y1248H.

A second c-Met inhibitor, PHA-665752, has also been described. This molecule inhibits c-Met-dependent activities *in vitro* and showed antitumor activity against c-Met-

dependent xenograft tumor models without side effects (43). Although this molecule does not have optimal drug-like properties, it provides proof of concept that inhibition of c-Met should result in tumor growth inhibition with an acceptable therapeutic index.

Which tumors are likely to respond to HGF- and c-Met-targeted drugs?

The preclinical evidence of a role for HGF and c-Met in the pathogenesis of cancer is compelling. However, whether agents targeting the HGF/c-Met axis will be successful in the clinic remains to be determined. The difficulty will be to establish which types of cancers or which patients within a particular type of cancer are more likely to respond to these agents. Several clinical studies have demonstrated that overexpression of HGF and/or c-Met correlates with poor prognosis (http://www.vai.org/vari/metandcancer/index.aspx). Higher levels of HGF and/or c-Met are also often correlated with late stage and the presence of metastatic disease (44-47). The question at this point is whether overexpression of the ligand or the receptor will suffice to predict sensitivity to targeted therapeutic agents. As exemplified by the epidermal growth factor receptor (EGFR), simple overexpression may not predict sensitivity to blocking agents (48). This is perhaps not surprising, as later stage cancers are unlikely to depend on a single pathway for survival (49, 50). Nonetheless, a strong case can be argued that HGF and c-Met are important and perhaps essential for tumor maintenance or progression. Indeed, overexpression of HGF and/or c-Met has been detected in most types of human cancers (51-62). Moreover, in light of the well-documented roles of these proteins in cell migration and invasion, it is tempting to hypothesize that blocking their activity will have a major impact on many types of late-stage cancers, both on the primary lesion and on metastases. Proof of this intriguing concept will have to await results from ongoing and future clinical trials.

Perhaps a better indicator of tumor dependence on this axis would be amplification or activating mutations of the receptor. Indeed, a number of successful targeted therapies to date impact genes that are either amplified, such as *erbB2* for trastuzumab (63, 64), or that harbor activating mutations, such as *bcr-abl* for imatinib (65, 66). c-Met has been shown to be amplified in some tumors (44, 67, 68). Amplification of c-Met is relatively rare in most types of primary tumors, but appears more frequent in gastric cancer. c-Met amplification may lead to tumor addiction and therefore render these tumors very sensitive to inhibitors of this pathway (69). Moreover, as documented by Di Renzo *et al*., c-Met amplification may be much more frequent in metastases (44).

The ultimate proof that c-Met dysregulation causes cancer is best documented in type I hereditary papillary renal cell cancer (HPRCC) (24). These patients harbor germ line-activating mutations of *MET* and develop multiple, bilateral renal tumors. If left unchecked, these tumors can metastasize, but interestingly, these patients rarely or never develop primary tumors in other organs. One hypothesis for this localized form of cancer is that the kidney has relatively high levels of HGF and its processing protease, urokinase-type plasminogen activator (uPA) (27). This hypothesis stems from work demonstrating that epithelial cells overexpressing mutant c-Met required HGF for transformation and that blocking the HGF signal in the cells prevented transformation (27). This implies that these tumors are at least partially dependent on HGF perhaps for maintenance or progression. Moreover, Chiara *et al*. found that c-Met-activating mutations shift the balance toward a more active conformation of the kinase, but require a second hit, such as HGF, for full activation (70). This may explain the long latency of HPRCC tumors, which usually cause symptoms starting in the fourth decade of life, and their exclusive location in the kidney.

Several missense mutations have been found in HPRCC patients, some of which are homologous to mutations in *c-kit* that cause systemic mastocytosis and *RET* found in multiple endocrine neoplasia syndrome type 2B (MEN2B). While the majority of mutations were found in the TK domain, some mutations were also identified in the juxtamembrane domain and in the extracellular domain. Several studies have demonstrated that the TK mutations are transforming *in vitro* and oncogenic in animals, although the exact mechanism downstream of c-Met may differ from mutation to mutation (25, 26, 71-73). Much less data exist for mutations outside the TK domain. Juxtamembrane point mutations appear weaker than TK mutations and less oncogenic (74), while there is no evidence in animal models that the extracellular mutation identified by Kim *et al*. is oncogenic (75). Finally, deletion of exon 14 of *MET* has been reported in primary lung tumors (76). This mutation deletes the c-Cbl binding site in the juxtamembrane domain of c-Met, resulting in a more stable protein with longer lasting signaling upon HGF activation. This form of c-Met is tumorigenic in animals in the absence of ligand, but tumor growth can be further stimulated by activating the receptor, confirming *in vitro* findings that this mutant is ligand-dependent. Furthermore, signaling and cell proliferation mediated by the mutant receptor were repressed in the presence of a c-Met-antagonist antibody, suggesting that this type of mutation may respond to a c-Met or HGF inhibitor in the clinic.

Oncogenic somatic mutations of c-Met have also been found in several other types of cancers, including some cases of sporadic PRCC, liver, lung, ovarian and head and neck cancers (72, 74, 75, 77-82). These mutations are relatively rare and typically are found in < 10% of cases. However, the number of published studies reporting c-Met mutations is small and relatively few patients have been examined. It will be interesting to see whether c-Met oncogenic mutations will be more common as more tumor genomes are sequenced. It is unknown at this time whether these tumors will be ligand-dependent. In any case, these tumors are good candidates to test c-Met-targeted agents.

Whether inhibitors of HGF and/or c-Met will have an impact on many types of cancers that overexpress the

ligand/receptor or have a more limited use in tumors that have amplifications or oncogenic mutations, retrospective analysis of patient samples with reliable biomarkers will be useful to understand the basis of the antitumor activity of these agents. Indeed, the success of any targeted therapy is largely dependent on identifying the right patients to treat, to administer the optimal amount of drug and to understand the basis for the response to the agent being studied. A number of potential biomarkers can be exploited for HGF- and c-Met-targeted therapies, but their validity will need to be studied in the clinic. These include the assessment of expression levels for ligand and receptor, the presence of amplifications and the identification of mutations. Knowledge of these different parameters may ultimately aid in the identification of patients whose tumors are dependent on the HGF/c-Met pathway.

Diversifying the toolbox

The arsenal of cancer drugs is growing and so are the choices for single-agent and combination therapies. In some patient populations where the actions of an oncogene are dominant, a targeted drug given as a single agent can be very effective. This is the case with imatinib in chronic myelogenous leukemia (CML) and could also be the case for cancer patients with activating c-Met mutations taking HGF/c-Met targeted agents. On the other hand, HGF/c-Met-targeted agents will also have to be tested in combination with other drugs, since the majority of tumors harbor multiple genetic lesions and are likely dependent on several pathways. It will be interesting to uncover the potential benefits of intervening in the tumor microenvironment by blocking HGF paracrine activity while attacking tumor cells directly with a second drug. The outcome of clinical trials combined with the molecular analysis of individual tumors will help to define the best way to use HGF/c-Met drugs to benefit patients.

Conclusions

Cancer is a devastating disease and is likely to remain prominent as the population ages. Even though overall survival for the major types of cancer has not improved in the last few decades, many new therapies have given hope to thousands of patients. New tools, including targeted drugs and biomarkers, are being discovered and developed that will certainly impact cancer treatment in the future. Whether the future holds more cures for cancer patients in still unknown, but it is conceivable that cancer will increasingly become a disease that people live with rather than die from.

Within the growing arsenal of cancer drugs, HGF- and c-Met-targeted drugs offer potentially effective new therapies that will give more treatment options for cancer patients. The promise of emerging drugs against this pathway awaits clinical validation, but for now, the evidence that HGF and c-Met are important drug targets gives us a path to pursue and hope for the future.

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