MET Pathway as a Therapeutic Target

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Dysregulation of mesenchymal-epithelial transition factor receptor tyrosine kinase pathway leads to cell proliferation, protection from apoptosis, angiogenesis, invasion, and metastasis. It can be dysregulated through overexpression, constitutive activation, gene amplification, ligand-dependent activation or mutation. New drugs targeting various mesenchymal-epithelial transition factor pathways are being investigated with promising results.

Key Words: MET pathway, Therapeutic target, Thoracic tumorigenesis.

(*J Thorac Oncol.* 2009;4: 444–447)

Many novel pathways critical to thoracic tumorigenesis are being identified and developed as potential therapeutic targets. Mesenchymal-epithelial transition factor (MET) receptor tyrosine kinase can be mutated or overexpressed in a number of epithelial human cancers, including lung and mesothelioma. It is activated by its ligand hepatocyte growth factor (HGF) and in malignant cells triggers a number of intracellular signaling transduction pathways resulting in alteration of biologic functions including metastasis.[1–3]

In this pathway review, we will highlight the basic biology of MET and current approaches to therapeutic targeting.

MET Structure and Pathway

MET was first discovered as an oncogene that encodes for the tyrosine kinase receptor for HGF. The gene for MET is located on chromosome 7q21-q31 and encodes for a single precursor that is posttranscriptionally digested and glycosylated, forming a 50 kDa extracellular α-chain and a transmembrane 140 kDa β-chain, which are linked by disulfide bonds. The MET β-chain contains homologous domains shared with other proteins, including a semaphorin (sema) domain, a PSI domain (in plexins, semaphorins and integrins), four IPT repeats (in immunoglobulins, plexins and transcription factors), a transmembrane domain, a juxtamembrane domain, a tyrosine kinase (TK) domain, and a carboxy-terminal tail region.[4,5]

MET's ligand has been identified as HGF which is secreted by fibroblasts and smooth muscle cells.[6] It binds MET's sema domain and induces MET dimerization, autophosphorylation, and activation of tyrosine kinase catalytic activity.[7,8] Tyrosine phosphorylation of JM, TK and tail domains respectively regulate internalization, catalytic activity, and docking of substrates such as Gab-1, Grb2, Shc, c-Cbl, which subsequently activate signal transducers such as PI3Kinase, PLC-γ, STAT, ERK1, ERK2, and FAK (Figure 1). Gab-1 is MET's unique adaptor protein which mediates numerous MET-initiated signals.[9–14] Gab-1 activates both the Erk and PI3K pathways. The Erk pathway regulates mitogenesis and the PI3K pathway regulates cell survival through the Akt/PKB pathway. Both pathways mediate cell adhesion, motility and invasion.[15–17] Cell migration and invasion are mediated by Ras, Crk, and c-src/FAK, and branching morphogenesis further requires the STAT3 and PLC-γ pathways.[18–22] Specifically, activation of Ras-Rac1/Cdc42-PAK and Gab1-Crk-C3G-Rap1 regulates cell adhesion and cytoskeletal proteins. Downstream molecules involved in the regulation of MET-induced motility and migration include cadherins, integrins, focal adhesion kinase, and paxillin.[23] Of note, paxillin can be somatically mutated in lung cancer, and is an important downstream target of MET.[24] Furthermore, MET signaling is involved in the regulation of tumor angiogenesis, either directly, through the proangiogenic activity of HGF that induces the formation of new vessels and the sprouting of the preexisting ones, or indirectly, through the regulated secretion of angiogenic factors, such as vascular endothelial growth factor A, interleukin-8, and thrombospondin-1.[25–28]

MET in Cancer

The regulation of MET can be influenced through overexpression, constitutive kinase activation, gene amplification, mutation, or paracrine/autocrine activation through HGF.[29,30] It is previously shown that 67% of adenocarcinomas, 60% of carcinoids, 57% of large cell carcinomas, 57% of squamous cell carcinomas, and 25% of small cell lung cancer (SCLCs) strongly expressed MET.[31] When assessing for functional activity with p-MET staining, 44% of adenocarcinomas, 86% of large cell, 71% of squamous cell, 40% of carcinoids, and 100% of SCLCs demonstrated MET phosphorylation at the Y1003 c-Cbl binding site; 33% of adenocarcinomas, 57% of large cell and 50% of

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Disclosure: The authors declare no conflicts of interest.

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ISSN: 1556-0864/09/0404-0444

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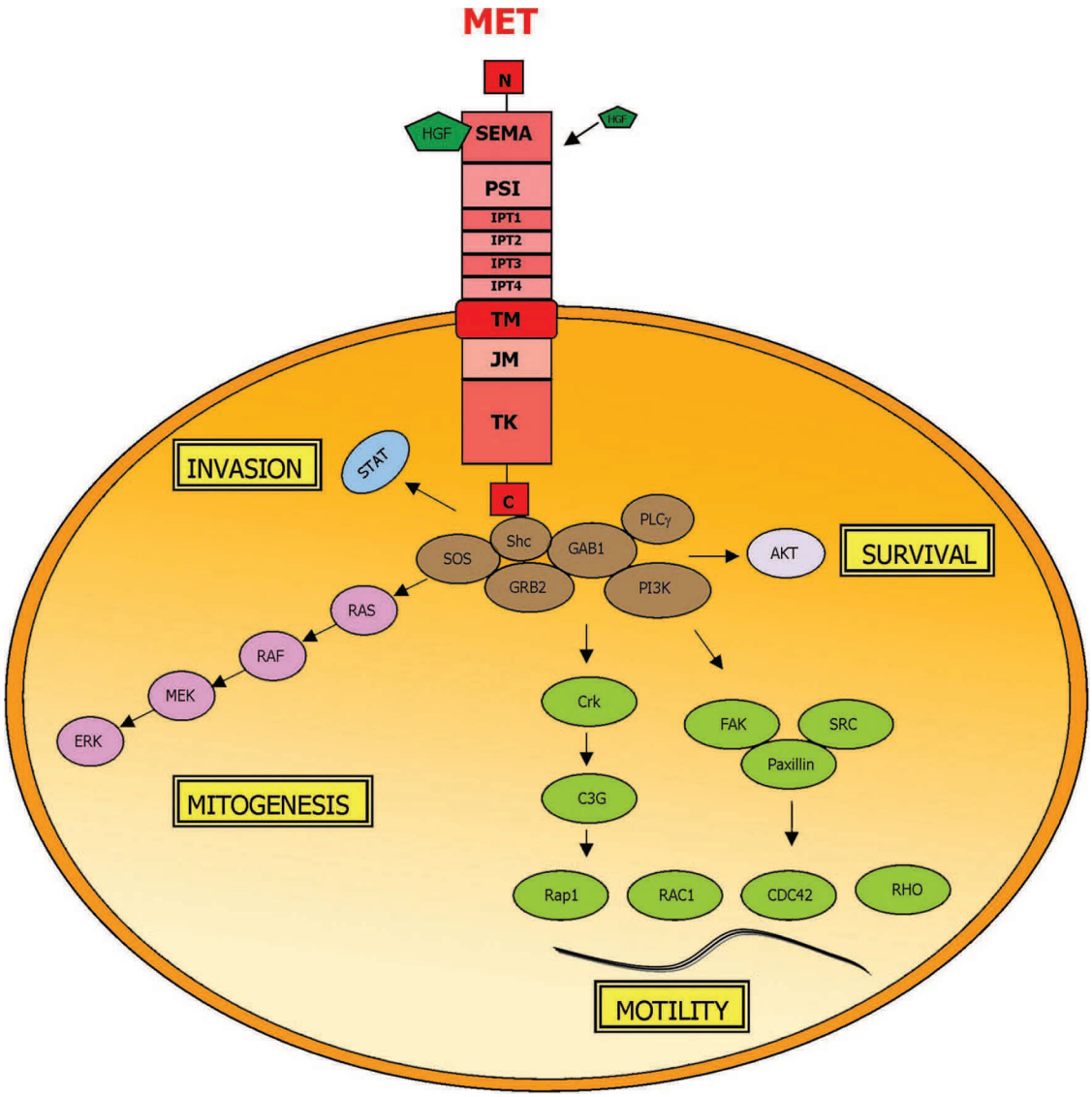

FIGURE 1. Mesenchymal-epithelial transition factor (MET) Structure and Pathway. HGF, hepatocyte growth factor; SEMA, Semaphorin-like; PSI, found in Plexins, Semaphorins, and Integrins; IPT, found in Ig-like regions, Plexins and Transcription factors; TM, Trans-Membrane; JM, Juxta-Membrane; TK, Intracellular Tyrosine Kinase; SOS, son of sevenless; GRB2, growth factor receptor-bound protein 2; GAB1, GRB2-associated binding protein 1; PI3K, phosphoinositol 3 kinase; PLCγ, phospholipase Cγ.

SCLCs demonstrated MET autophosphorylation at the Y1230/1234/1235 site.[31]

A large number of missense mutations occur in the JM domains which are thought to be key regulators of receptor tyrosine kinases catalytic functions. Mutations at R988C, T1010I, and S1058P were identified in a study of 127 lung adenocarcinoma.[31] Among these JM domain mutations, R988C and T1010I were previously found to enhance tumorigenicity, MET/downstream molecule phosphorylation and cell motility in SCLC.[32] Mutations in the sema domain affect binding to HGF and those in the TK domain constitutively activated MET protein in hereditary papillary renal cell carcinomas.[33] Besides missense mutations, MET-mediated tumorigenesis could be a result of gene amplification, leading to receptor overexpression. More recently, MET amplification was observed in approximately 20% of lung cancer specimens with acquired resistance to the epidermal growth factor receptor inhibitors.[34,35] It is shown that amplification of MET causes gefitinib resistance by driving HER3-dependent activation of PI3K. Conversely, inhibition of MET signaling restored sensitivity to gefitinib.[34] This observation further strengthens the hypothesis that MET activation contributes critically to tumor cell resistance.

Amplification of focal adhesion signaling molecules such as paxillin has also been demonstrated in lung cancer. Paxillin was highly expressed (compared with normal lung), amplified (12.1%, 8 of 66) and correlated with increased MET and epidermal growth factor receptor gene copy numbers, or mutated (somatic mutation rate of 9.4%, 18 of 191).[24]

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Therapeutics

It is anticipated that targeted therapy against MET and its pathway will lead to significant inhibition of cancer growth and metastasis. The expression of MET protein has been targeted at the RNA levels with small interference RNA, microRNA, MET-specific ribozymes or at the level of protein maturation. Suppression of MET expression by delivering small interference RNA is a novel approach. SiRNA binds to ribosomes in place of MET RNA, effectively silencing MET RNA. MicroRNA is a form of single-stranded RNA that is thought to regulate gene expression by cleaving specific mRNA or by pairing with target mRNAs to silence their translation.[36,37] Ribozymes are RNA-based enzymes that bind to and cleave RNA molecules in a sequence- specific manner. MET protein expression can be targeted at the level of protein maturation through inhibition of the heat shock protein (HSP90) by geldanamycin or members of the anisomycin antibiotic family.[38]

NK (N-terminal hairpin domain and Kringle domain) inhibitors form a family of four variants of HGF α-chain. NK4, a variant of HGF comprising only the four-kringles of the α-chain is a promising competitor for HGF. NK4 binds to MET without inducing receptor activation and thus behaves as a full antagonist.[39] Moreover, as a consequence of its structural similarity to angiostatins, but independently from its effect on MET signaling, NK4 is able to inhibit angiogenesis induced by vascular endothelial cell growth factor (VEGF) and basic fibroblast growth factor.[40] Similarly, the anti-HGF antibody binds an epitope in the β-chain of HGF and prevents it from binding to MET. In preclinical studies, this AMG102 (Amgen, Inc), a fully humanized monoclonal anti-HGF IgG showed good pharmacokinetic and safety profiles in cynomolgus monkeys[41] and synergism with temozolomide and docetaxel in a U-87 MG (human glioblastoma derived containing HGF/MET autocrine loop cells) xenograft model in vivo.[42] Phase I clinical trial with AMG 102 has been completed and phase II trials are currently being designed.

Several MET inhibitors are currently under investigation. Previously, a broad-spectrum kinase inhibitor at ATP binding site, K252a, was identified.[43] Efforts to develop more specific inhibitors have led to characterization of SU11274 and PHA665752. At nanomolar concentrations, they are both at least 50-fold more selective for MET compared with other receptor tyrosine kinases and strongly inhibit HGF-induced activation of MET in cultured cells and tumorigenicity in mouse models.[25,44,45] Most recently, PF2341066, an orally available selective competitor for MET has been shown to inhibit tumor cell growth in vitro and in vivo.[46,47]

There are a number of kinase inhibitors that have reached clinical trials.[48] These include PF2341066, XL880 (Exelixis), XL184 (Exelixis), ARQ197 (ArQule Inc.), SGX523 (SGX Pharmaceuticals), and MGCD265 (MethylGene). SGX523 had to be stopped prematurely in phase I trial due to unexpected renal toxicity. Many of these inhibitors also have activity against other kinases. In the future, differentiation of MET inhibitors into specific kinase targets will need to be made. Determining specific patient subsets based on genetic profile that are more likely to respond to MET kinase inhibitors will contribute to better clinical outcome of these inhibitors. Lastly, as many tumors may require inhibition of more than one pathway, combinational strategies will need to be further explored.

ACKNOWLEDGMENTS

Supported, in part, by NIH/NCI, American Lung Association, V-Foundation (Guy Geleerd Memorial Foundation), Kate McMullen Foundation, Respiratory Health Association of Chicago, and Mesothelioma Applied Research Foundation (Jeffrey P. Hayes Memorial Grant).

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