The Biology Behind

Suppression of Met Expression: A Possible Cancer Treatment

Commentary re: S. J. Kim *et al.*, Reduced c-Met Expression by an Adenovirus Expressing a c-Met Ribozyme Inhibits Tumorigenic Growth and Lymph Node Metastases of PC3-LN4 Prostate Tumor Cells in an Orthotopic Nude Mouse Model. Clin. Cancer Res., *14:* 5161-5170, 2003.

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Introduction

Met is a receptor protein tyrosine kinase and the only known receptor for HGF/SF.[2] This ligand/receptor signaling pair mediates a vast range of biological activities not only in normal organ development and physiological functions but also in tumor proliferation, progression, invasion, and metastasis. Tumor cells that express high levels of Met molecules on their surface are more malignant and metastatic. In many carcinomas, HGF/SF acting in a paracrine manner is produced by stromal cells adjacent to the tumor. Inhibition of Met expression suppresses the malignant progression of tumor cells and has been the subject of a growing number of studies. Abounader *et al.* (1) were first to use a ribozyme strategy to suppress the growth of human glioblastoma tumors. Because overexpression of Met receptors is observed in a wide spectrum of carcinomas and considered to play a key role in the progression of cancer cells, targeting of this molecule could become one of the most useful treatment modalities for refractory cancers. Molecular targeting of the Met signaling pathways by using specifically designed genes, which target *c-met,* can be used as a treatment modality for controlling tumor growth and metastasis. In this issue, Kim *et al.* (2) report that an adenovirus expressing c-Met ribozyme inhibits tumorigenicity and lymph node metastasis of human prostate cancer cells by using an orthotopically implanted *in vivo* mouse model. In prostate cancer cells especially, high expression of Met is associated with resistance against chemotherapy including hormonal therapy and is often observed in the advanced stages of clinical cases. By reducing Met expression using a ribozyme that targets Met mRNA, tumor growth and lymph node metastasis were dramatically inhibited.

Overview of the HGF/SF-Met Signaling Pathways. The Met receptor was discovered first as an oncogene and then as a tyrosine kinase receptor proto-oncogene of which the ligand was at first unknown (3, 4). Subsequently, Met was recognized as the receptor to HGF (5). HGF and SF were discovered independently as a growth factor for hepatocytes (6, 7) and as an effector for the movement of epithelial cells (8, 9). Molecular cloning, sequence, and functional analyses revealed that these pleiotrophic activities are derived from one common protein (10, 11) named HGF/SF.

Met is synthesized as a single peptide and cleaved into a disulfide-linked heterodimer made of α- (M_r 45,000) and β-subunits (M_r 145,000). The Met α chain is located outside the membrane, whereas the β chain consists of an extracellular domain, a single transmembrane domain, and a cytoplasmic moiety in which the receptor tyrosine kinase domain resides. Upon binding the HGF/SF ligand to the extracellular domain of Met, downstream cytoplasmic signal transduction pathways are activated (Fig. 1). This activation is mediated through a multidocking site (12), which can bind signaling molecules and adaptor proteins such as Src, Gab1, Grb2, Shc, Shp2, SOS, phosphatidylinositol 3-kinase, and phospholipase C γ (13–15). In general, recruitment and activation of these molecules induces activation of the Ras-Rac/Rho pathways, the Ras-mitogen-activated protein kinase pathway, the signal transducers and activators of transcription 3 and Akt pathways. The Akt pathway inhibits apoptosis and, therefore, contributes to HGF/SF activity as a survival factor (16). The Met signaling induces cell growth and motility, but HGF/SF is also a potent angiogenic factor (17–19). Signal transducers and activators of transcription 3 serves to enhance anchorage independence and tumorigenicity of cancer cells (20).

Like Met, HGF/SF is synthesized and secreted as an inactive precursor, a 728 amino acid single chain with a 29 amino acid signal peptide. The ligand must be proteolytically cleaved into a disulfide-linked α chain (M_r 69,000) and β chain (M_r 34,000) to be biologically active (21). HGF/SF can be regulated at the transcriptional level as well as in tissues after it has been produced and secreted mainly from mesenchymal cells. HGF/SF binds to heparan sulfate proteoglycans with a very high affinity (nanomolar; Ref. 22). Tissues abundant in heparan sulfate proteoglycans retain HGF/SF for release in response to inflammatory signals for activation of epithelial cells (23). The level of active HGF/SF can also be regulated by proteolytic processing of the inactive single chain polypeptide, which enables pro-HGF/SF to be biologically active. Several serine proteases, such as urokinase plasminogen activator, tissue plasminogen activator, and other coagulation factors are able to convert pro-HGF/SF into active HGF/SF (24). Although pro-HGF/SF can bind to Met, it cannot activate the receptor signaling pathways (25).

In the mid 1990s, phenotypes of Met-HGF/SF knockout animal models were studied, and homozygous null animals were shown to be embryonic lethal for both ligand and receptor. Loss

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[2] The abbreviations used are: HGF, hepatocyte growth factor; SF, scatter factor; TSP, thrombospondin; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; RNAi, RNA interference.

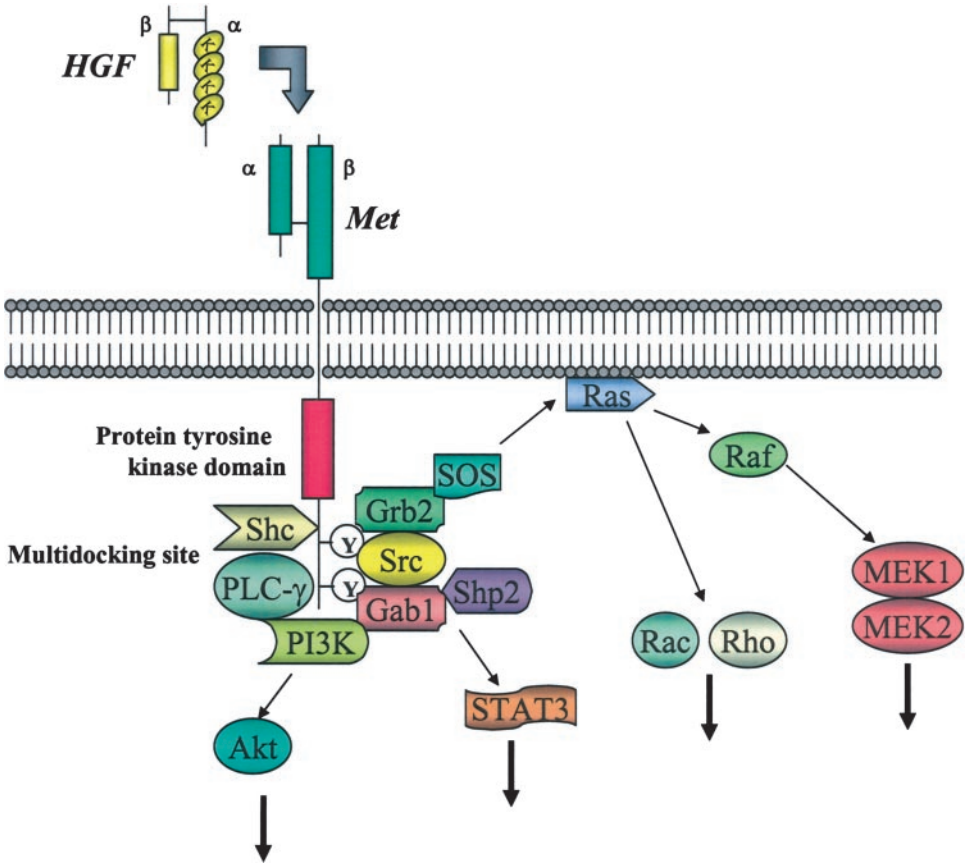

Fig. 1 HGF/SF, the Met receptor and downstream signaling pathways.

of Met expression results in embryonic lethal because of hypoplasticity of placenta (26), disturbance of liver development (27), and malformation of the limb buds and diaphragm (28). In addition, HGF/SF-Met signaling is involved in axon guidance and participates in the formation of the central nervous system (29). HGF/SF and Met knockout animals show identical phenotypes, providing unequivocal evidence that Met is the only functional receptor for HGF/SF (15, 30). In addition to the embryological processes, HGF/SF and Met are expressed broadly in adult tissues. After tissue damage, a rapid increase in the plasma levels of HGF/SF is observed, and subsequently increased ligand expression is found in the injured tissue or organs. Tissue regeneration process in the main organs, such as heart, kidney, and liver, is closely related to HGF/SF expression (31–33). Therapeutic treatment with HGF/SF has been shown to enhance tissue regeneration (34).

Role of the Met Protein in Tumor Progression, Invasion, and Metastasis. Besides the physiological and pathophysiological functions, HGF/SF-Met signaling has been shown to play an important role in the malignant transformation of tumor cells. In response to HGF/SF stimulation, cells expressing Met are more proliferative and scatter (increased motility), and become more invasive and undergo branching morphogenesis in the presence of extracellular matrices (35). *In vivo*, HGF/SF induces angiogenesis by stimulating the production of VEGF and inhibiting TSP-1 expression (19). When tumor cells undergo the metastatic process, loss of cell-to-cell contact and reorganization of adhesion molecules is considered to be essential (36, 37). Met mediates all of the tumor progression activities, proliferation, invasion, survival, metastasis, and angiogenesis (38). Thus, *met* is one of the most important cancer genes. Activating mutations in Met have been found in several types of cancers, but activation of Met in most cancers is believed to occur through ligand-dependent autocrine or paracrine mechanisms (39, 40).

Animal models have helped to understand the role of HGF/SF-Met signaling in the progression, invasion, and metastasis of tumor cells (38, 41, 42). Cell lines that ectopically overexpress HGF/SF and/or Met are tumorigenic and metastatic (43–45). Tumor cells implanted into HGF/SF transgenic mice display enhanced tumor growth and metastasis (46, 47). In addition, HGF/SF transgenic mice develop a broad array of histologically distinct tumors of both mesenchymal and epithelial origin (48). Because most neoplasms arising from HGF/SF transgenic mice demonstrate overexpression of both the HGF/SF transgene and endogenous *met*, and have an enhanced Met kinase activity, autocrine signaling contributes the promotion of tumorigenesis.

Invasive capacity of tumor cells derived by HGF/SF stimulation is regulated by an increased level of MMPs and urokinase plasminogen activator (49, 50). The urokinase plasminogen proteolysis network activated by Met-HGF/SF signaling is

closely coupled with the signal transduction pathway of the proteases and forms a positive feedback cascade (24). Production of proteases in concert with cell motility is an essential factor for cell migration through the extracellular matrix. Branching morphogenesis that is mediated by Met-HGF/SF signaling also depends on this process, and plasmin-dependent branching is inhibited by both inhibitors of plasmin and MMPs (51).

HGF/SF-Met signaling also stimulates the production of VEGF and induces angiogenesis. This neovascularization is essential for tumor cell growth (52). Using human endothelial cells and vascular smooth muscle cells revealed that HGF/SF stimulates the expression of MMP-1, VEGF, Met, and HGF/SF itself (53). Up-regulation of these angiogenesis-related genes is largely because of *ets-1*, an essential transcription factor for angiogenesis. A recent report of hepatocarcinogenesis using HGF/SF transgenic mice showed that HGF/SF overexpression accelerated diethylnitrosamine-induced hepatocarcinogenesis and that tumors were often accompanied by abnormal blood vessel formation (54). Expression of VEGF was up-regulated in parallel with HGF/SF transgene expression. HGF/SF has been shown recently, in the same cell, to up-regulate VEGF and to shut off TSP-1 expression (19), and TSP-1 was shown to play a major role in inhibiting tumor vascularization. Therefore, HGF/SF helps to promote carcinogenesis through autocrine activation of the HGF-Met signaling pathway in association with stimulation of angiogenesis by HGF/SF directly working on endothelial cells, but also extrinsically by enhancing VEGF expression in tumor cells and endothelial cells, and down-modulating TSP-1.

Met Expression in Prostate Carcinoma Cells. Prostate cancer is the most common form of cancer other than skin cancer among men in the United States. It is second leading cause of cancer-related death among men after lung cancer. The American Cancer Society estimates that in 2003, ~220,900 new cases of prostate cancer will be diagnosed, and 28,900 men will die of the disease. Therefore, the importance of early diagnosis and treatment of this cancer has been increasing.

In the normal prostate, Met is expressed predominantly by prostate epithelial cells, whereas HGF/SF is synthesized by prostate stromal cells (55). HGF/SF causes growth inhibition, sustained phosphorylation of mitogen-activated protein kinase, and increased expression of low molecular weight cytokeratins, such as CK18, suggesting that the HGF/SF-Met pathway is involved in cell differentiation in normal prostate epithelial cells (55). Met is also expressed in localized and metastatic prostate cancers. However, unlike normal prostate epithelial cells, HGF/SF significantly stimulates the proliferation of prostate cancer cells (55) with the exception of PC3 cells.[3] A clear relationship between Met protein expression and high-grade adenocarcinomas has been reported (56). Moreover, the incidence of bone metastases is closely related to the expression level of Met (57), and virtually all bone metastases are strongly Met positive. Because HGF/SF is expressed by human prostatic stromal myofibroblasts in primary culture but not by most human prostatic carcinoma cell lines (58), the growth and progression of prostate cancer depends on the stromal-epithelial interaction, which is under the control of paracrine mechanism.

Two distinct prostate cancer cell groups have been reported; one is androgen-dependent (responsive) carcinoma (*e.g.*, LNCaP) and the other is androgen-independent (unresponsive) carcinoma (*e.g.*, DU 145, PC-3, and ALVA-31; Refs. 59, 60). Generally, androgen-independent prostate cancer is resistant to therapy and is often metastatic (61, 62). A recent report suggests that expression of Met is associated with androgen-insensitive prostate cancer (60). In addition, HGF/SF is expressed in interstitial cells, especially in hormone-treated cancer tissue, indicating that the growth factor pathway changes with the hormonal status (63). *c-met* transcripts were identified in two androgen-insensitive human prostatic carcinoma cell lines (DU 145 and PC-3) but not the androgen-sensitive LNCaP cell line (58). In our laboratory very low levels of Met are expressed in LNCaP (64), and whereas PC3 cells express high levels of Met, they are generally unresponsive to HGF/SF. This may be because of ligand-independent Met activation (65), yet another form of Met activation. HGF/SF-Met signaling pathway may affect the androgen insensitivity. Intermediate cells in the prostate gland have been proposed as targets of malignant transformation in prostate cancer and precursors of androgen-independent tumor progression. Actually, in prostate cancer tumor cells, intermediate cells have been shown to display high Met levels (66).

In an animal model using testosterone stimulation, HGF/SF and Met were reported to be coexpressed in the lesions of prostatic dysplasia and carcinomas (67). This suggests that an autocrine mode of action may be involved in carcinogenesis of some types of prostate cancers.

Molecular Targeting against the HGF/SF-Met Pathway. Efforts to suppress Met expression in tumor cells has been studied extensively and, in general, reduction of Met expression or HGF/SF activity leads to the inhibition of tumorigenicity, invasive activity, and metastatic potential of tumor cells. Molecular targeting of c-*met* or Met by way of chimeric transgenes consisting of U1 small nuclear RNA, a hammerhead ribozyme, and antisense sequences against *c-met* is one approach. Reduction of Met suppresses the invasiveness of breast cancer cells (68), and tumorigenicity and tumor growth of glioblastoma cells (1). These results suggest that targeting the HGF/SF-Met signaling pathway may be an important approach in controlling tumor progression. By applying a similar technique, Met suppression also suppresses metastatic growth of colon tumor cells in the liver (69). Moreover, combination of anti-HGF/SF and anti-*c-met* ribozymes showed remarkable suppression of tumor growth and angiogenesis, as well as promotion of apoptotic cell death of glioblastoma cells (70). Because Met activation may play a key role in malignant progression of many cancers, targeting of Met by using a ribozyme system would be a possible therapeutic way to control cancer cells (2) if the molecules can be directed specifically to the tumors.

Abrogation of the HGF/SF-Met signaling pathway using dominant-negative forms of the Met receptors that can bind HGF/SF but not transduce the signals to downstream molecules also suppresses tumor growth and their ability to form lung metastases *in vivo* (71, 72). In addition, dominant-negative mutants of both human and murine Met can inhibit HGF/SF-

[3] N. Shinomiya and G. F. Vande Woude, unpublished observations.

mediated Met signaling and cell invasion of *ras*-transformed cells, suggesting that Met is involved in *ras*-mediated tumor progression (71).

Development of neutralizing anti-HGF/SF antibodies is one of the best ways to substantially disrupt the Met stimulation signals. Recent analysis revealed that growth of human glioblastoma xenografts expressing Met and HGF/SF were markedly reduced in the presence of HGF/SF-neutralizing monoclonal antibodies. Interrupting autocrine and/or paracrine Met-HGF/SF signaling in tumors dependent on this pathway is a possible intervention strategy (73).

A subunit of HGF/SF containing the four kringle-domains of the ligand (NK4) has been shown to effectively compete with HGF/SF. NK4 protein has been shown to inhibit tumor invasion, metastasis, and angiogenesis in a wide variety of tumor cells (74–77). However, this technique is only applicable to ligand-dependent cancers, and the effect is not uncertain in cancers expressing the constitutively activated form of Met protein.

Recent discovery of RNAi mediated by short interfering RNA has generated a new gateway to cancer therapy. RNAi has been found to have long-term gene knockout effects resulting from a post-transcriptional gene silencing mechanism that may involve the homologous recombination between intracellular mRNA and the mRNA components of an RNAi construct (78). Our recent experiments using an adenovirus vector expressing *c-met* short interfering RNA reveal strong suppression of tumor growth, scattering, and invasion activity in breast and prostate cancer cells.[4]

New Paradigm of Cancer Treatment. Because HGF/SF-Met signaling is involved in important biological behavior of tumor cells including proliferation, migration, invasion, metastasis, and angiogenesis, targeting of this pathway would seem to provide an ideal means for controlling malignant tumor growth. Molecular targeting of Met can convert the tumor cells from malignant to benign and attenuate the aggressive cancer cell behavior. Suppression of Met not only retards tumor progression, but also suppresses the production of VEGF that affects neovascularization (52–54).

Development of the drugs that can suppress Met function is another approach. Certain members of the geldanamycin family of ansamycin antibiotics have been found to act as potent inhibitors of HGF/SF-Met pathways. The geldanamycins at nanomolar concentrations down-regulate Met protein expression by inhibiting heat shock protein 90 function and interfering with the chaperone activity (79). In addition, geldanamycins at femtomolar concentrations display inhibitory properties on HGF/SF-Met-induced plasmin activation, which is important in tumor invasion and metastasis. A geldanamycin derivative, 17AAG, is in clinical trials and may have important therapeutic potential for the treatment of cancers in which Met activity contributes to the invasive/metastatic phenotype. Actually, geldanamycin reduces the growth and viability of lung cancers by causing apoptosis (80).

As mentioned above, HGF/SF and Met are expressed in many normal organs and involved in the maintenance of normal physiological functions. Therefore, when we think about a target gene therapy in the actual clinical situation, how the tumor-specific delivery will be conducted is a major problem that still remains to be solved.

[4] Manuscript in preparation.

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