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HGF/SF-Met Signaling in the Control of Branching Morphogenesis and Invasion

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Abstract Hepatocyte growth factor/Scatter factor (HGF/SF) is a multifunctional growth factor which can induce diverse biological events. In vitro, these include scattering, invasion, proliferation and branching morphogenesis. In vivo, HGF/SF is responsible for many processes during embryonic development and a variety of activities in adults, and many of these normal activities have been implicated in its role in tumorgenesis and metastasis. The c-Met receptor tyrosine kinase is the only known receptor for HGF/SF and mediates all HGF/SF induced biological activities. Upon HGF/SF stimulation, the c-Met receptor is tyrosine-phosphorylated which is followed by the recruitment of a group of signaling molecules and/or adaptor proteins to its cytoplasmic domain and its multiple docking sites. This action leads to the activation of several different signaling cascades that form a complete network of intra and extracellular responses. Different combinations of signaling pathways and signaling molecules and/or differences in magnitude of responses contribute to these diverse series of HGF/SF-Met induced activities and most certainly are influenced by cell type as well as different cellular environments. In this review, we focus on HGF/SF-induced branching morphogenesis and invasion, and bring together recent new findings which provide insight into how HGF/SF, via c-Met induces this response. J. Cell. Biochem. 88: 408–417, 2003.

Key words: HGF/SF; Met tyrosine kinase; branching morphogenesis; invasion

HGF/SF AND MET RECEPTOR TYROSINE KINASE

Hepatocyte growth factor (HGF), originally identified as a mitogen for hepatocytes [Nakamura et al., 1989] was subsequently shown to be identical to Scatter factor (SF), a ligand with a dramatically different activity of inducing epithelial cell dissociation ("scattering"). Indeed the SF was selected for uniquely lacking mitogenic activity [Stoker et al., 1987; Rosen et al., 1989]. HGF/SF is therefore a unique growth factor that elicits multiple cellular responses including mitogenesis, motility and morphogenesis. Interestingly, HGF/SF is produced primarily by mesenchymal cells and is secreted as pro-HGF/SF, an inactive single chain precursor molecule which is activated by proteolytic cleavage into disulfide-linked α and β chains [for review see Birchmeier and Gherardi, 1998; Trusolino and Comoglio, 2002]. The high affinity receptor for HGF/SF is the receptor tyrosine kinase c-Met [Bottaro et al., 1991]. Met was first identified as an activated oncogene as a result of a chemically induced chromosomal rearrangement [Cooper et al., 1984; Park et al., 1986]. Met is synthesized as a polyprotein and is proteolyctic into cleaved α and β subunits as it is activated (matures) on the cell surface. In contrast to HGF/SF and in a paracrine manner, Met is predominantly expressed in the cells derived from the epithelial or endothelial origins [for review see Birchmeier and Gherardi, 1998]. HGF/SF-Met signaling is key in many developmental processes including promoting migration of muscle precursor cells and motoneurons [for review see Birchmeier and Gherardi, 1998].

Through Met, HGF/SF induces a wide spectrum of biological events, including scattering, invasion, proliferation, branching morphogenesis, transformation and angiogenesis [for reviews see Vande Woude et al., 1997; Furge et al., 2000; Comoglio, 2001]. Binding of HGF/

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SF to the extracellular domain of Met triggers phosphorylation of tyrosine residues in the kinase domain of Met. Once activated, Met elicits intramolecular phosphorylation of two critical tyrosine residues referred to as the multiple docking sites (Y^{1349}VHV and Y^{1356}VNV). These sites are located in the C-terminal cytoplasmic domain and serve to recruit downstream signaling molecules and adaptor proteins [for review see Furge et al., 2000; Comoglio, 2001] thereby amplifying the cellular responses from HGF/SF ligand to a multiple distinctive pathways. While HGF/SF-Met signaling plays a significant role during normal development [for review see Birchmeier and Gherardi, 1998], this signaling pathway has been implicated in the pathogenesis of most types of human solid tumors. This has sparked intensive efforts to identify or characterize the signaling molecules downstream of Met responsible for its role in malignant disease and important information has been gained from molecular studies and/or mouse models. This review brings recent observations on HGF/SF-Met signaling mediated branching morphogenesis and invasion, and discussion of how HGF/SF via Met can induce such diverse cellular events.

HGF/SF-MET SIGNALING INDUCED BRANCHING MORPHOGENESIS AND INVASION

Among many biological activities, branching morphogenesis and invasion appear to be the most notable and complicated cellular events induced by the HGF/SF. Branching morphogenesis is a crucial event for pattern formation in many organs and tissues during different development stages, including placenta, kidney, lung, mammary glands, and nervous systems, as well as for endothelial cells contributing the blood vessel network formation during tumor angiogenesis. Branching morphogenesis is typically mediated through changes in cell shape, asymmetric polarization of the cells in the direction of branching, branch elongation, cell–cell contact, cell-extracellular matrix (ECM) communication, ECM remodeling, controlled proteolysis (basement membrane matrigel or collagen invasion) and cell motility. Branching morphogenesis does not appear to occur in a random fashion and raise questions such as does the gradient of HGF/SF determine branch direction and how does HGF/SF–Met influence cytoskeletal reorganization specifically at the growing end of the branch? In many ways this in vitro activity could mimic in vivo morphogenesis [Vande Woude et al., 1997]. HGF/SF is one of the very few factors that can induce branching morphogenesis. In response to HGF/SF, Met expressing cells form branches in three-dimensional matrigel or tubule-like structures (tubulogenesis) in collagen gels [Montesano et al., 1991; Jeffers et al., 1996]. HGF/SF also induces cell proliferation and one fundamental question is whether proliferation is required for branching by HGF/SF. HGF/SF induces axon outgrowth of sensory neurons, and in this case, cell proliferation is not required because the axon is extended and elongated from a single neuron to reach other cells to form synapses. However, we speculate that cell proliferation may be required in HGF/SF-induced tubulogenesis or branching morphogenesis since these structures are multicellular and therefore cells at the tip of the branch would be expected to undergo cell division to elongate the branch structure. We hypothesize that the tip cells remain undifferentiated and proliferate, while the cells within the branch differentiate as influenced by cell–cell contact and cell–ECM interactions. However, it is not clear at the molecular level how branching is stimulated by HGF/SF. Curiously, we observe branching in response to HGF/SF, not only with carcinoma cell lines of epithelial origin, but also with sarcoma cell lines of mesenchymal origin.

Tumor cells invade adjacent tissues or metastasize to remote sites, organs or tissues via lymphatics or blood vessels. Invasion requires changes in cell adhesion, cell–cell dissociation, cytoskeletal reorganization and motility as well as regulated proteolysis to invade through the ECM or basement membranes. Scatter factor was originally identified as a mesenchyme-derived motogen inducing epithelial cell scattering [Stoker et al., 1987] and many studies have been conducted to investigate how HGF/SF regulates the invasive process. Cells expressing Met show enhanced motility in the direction of HGF/SF and increased invasive behavior through matrigel. The invasive phenotype becomes more dramatic when the cells are autocrine for HGF/SF, as determined by the spontaneous or experimental metastasis assays in athymic nude mice [for review see Vande Woude et al., 1997].

The interaction between epithelia and stromal mesenchym is important both in normal development and malignancy. Under the normal condition, Met receptors are predominantly expressed on the surface of epithelial or endothelial cells, and signaling is paracrine with HGF/SF localized to stromal tissue and mainly synthesized by mesenchymal cells. HGF/SF has high affinity for ECM heparan sulfate proteoglycans (HSPGs) [Hartmann et al., 1998] that sequester the ligand and probably localize the action of HGF/SF only to the surrounding Met expressing cells. Although binding of HSPGs is not required for the activation of HGF/SF-Met signaling [Hartmann et al., 1998], it probably plays a very important role in determining normal and abnormal specificity in what cells (tissues) are able to respond. Aberrant activation in HGF/SF-Met signaling leading to invasion and metastasis is therefore not unlike cell migration activities during development. HGF/SF-Met signaling is essential for pattern formation in skeletal muscle, placenta and sensory neurons. Mice nullizygous for c-Met are embryonic lethal, the developing embryos display defects in placenta formation, lack limb muscles that are derived from dermamyotome migratory precursor cells and display defects in spinal nerve outgrowth and branching [for review see Birchmeier and Gherardi, 1998]. HGF/SF induced branching also plays a role in the development of the metanephros and uteric bud as well as mammary gland development [for review see Birchmeier and Gherardi, 1998]. HGF/SF shares features in common with another unique growth factor, fibroblast growth factor (FGF) that can also induce epithelial cell migration and branching [Metzger and Krasnow, 1999]. Mice with targeted disruption of the FGF10 locus fail to form lungs and only display a blind-ended trachea [Sekine et al., 1999]. HGF/SF also contributes to fetal lung development and synergizes with FGFs in lung epithelial branching [Ohmichi et al., 1998]. Surprisingly, lung branching morphogenesis occurs without cell proliferation and the entire tracheal system appears to be formed solely by cell migration and changes in cell shape [Metzger and Krasnow, 1999], which suggests, at least in some tissue types or under certain circumstance, cell proliferation may not be required for HGF/SF-induced branching morphogenesis. Targeted disruption of the FGF receptor, FGFR2, shows defects in labyrinthine placental development [Xu et al., 1998], which is similar to the placental phenotype of HGF/SF or Met null mutant [for review see Birchmeier and Gherardi, 1998]. Obviously, these two pathways are not functional redundant and given the similarities, it is curious how these pathways might synergize during lung and placenta formation and whether they activate similar downstream molecules or regulate the expression of different genes.

Under pathological conditions, inappropriate expression of HGF/SF and/or Met is found in all types of solid tumors and often correlates with poor prognosis [for review see Vande Woude et al., 1997; Trusolino and Comoglio, 2002]. Compelling evidence of Met's role in human cancer is based on the discovery of germline and/or somatic activating mutations found in human papillary renal cell carcinomas [Schmidt et al., 1997] and other cancers. The mutations are located predominantly in the kinase domain of Met and lead to constitutive activation of Met, increased invasive phenotypes and metastatic disease [Jeffers et al., 1998]. Activating mutations in Met have also been found in gastric cancer, hepatocellular carcinomas and head and neck squamous cell carcinomas [for review see Trusolino and Comoglio, 2002].

HGF/SF-Met signaling cascade is a crucial pathway for invasion and metastasis [Rong et al., 1994]. Targeting this signaling may find an effective way to intervene or prevent the malignant process. Activation of the receptor by HGF/SF recruits a group of downstream molecules and/or adaptor proteins to its multidocking sites (Y^{1349}VHV and Y^{1356}VNV) and activates diverse downstream pathways [for reviews see Furge et al., 2000; Comoglio, 2001]. The list of downstream molecules and adaptor proteins continue to grow and utilize the tyrosine residues in the docking sites differentially. Grb2 prefers to bind Y^{1356}, while other factors including Gab1, p85 PI3K, PLC-γ, Src and Shc associate with both Y^{1349} and Y^{1356} [for review see Furge et al., 2000; Comoglio, 2001]. Mutation of Y^{1356} uncouples Met from Grb2, the mediator for Ras/MAPK pathway, through association with SOS, the mutation also impairs the interaction of Met with Gab1, a large scaffold adaptor protein which recruits of several important substrates to activated Met. Gab1 also interacts with Grb2 through its SH3 domain at the carboxy-terminus and this

interaction stabilizes the association of Gab1 with Met [for review see Furge et al., 2000]. Mutations in both Y^{1349} and Y^{1356} residues of Met eliminate the recruitment of these downstream signaling molecules and adaptor proteins to the multiple docking sites of Met and abolishes almost all HGF/SF induced biological events, including invasion and branching morphogenesis [for review see Furge et al., 2000; Comoglio, 2001]. Despite the importance of these two tyrosine residues, there is also evidence showing that the multi-docking site of Met is dispensable for Met-mediated Ras signaling and cell scattering [Tulasne et al., 1999], suggesting other regions in Met are also critical for HGF/SF-induced biological activity. Is the sequence of activations in c-Met important or do different Met molecules in the same cell perform differently?

To address some of these problems, animal models have been created with different Met mutations within the multi-docking sites using gene-targeting technology. Maina et al. generated a series of mice possessing different Met mutants with selectively optimal binding sites for PI3 kinase, Src or Grb2 [Maina et al., 2001]. Coupling Met specifically to PI3K or Src does not rescue the phenotypes of embryo lethality, nor defects in the migration of myoblast precursors as seen in loss-of-function Met mutant. However, germline mutations which are optimized for Src binding (but not PI3K) in the docking site of Met still allows for the development of normal placenta which supports the embryo until birth [Maina et al., 2001]. By contrast, mutations which select for PI3K binding (but not Src) in the docking sites allows for axonal outgrowth and branching [Maina et al., 2001]. Uncoupling Grb2 from Met leads to reduction of myoblast proliferation, but does not affect myoblast migration, branching of sensory neurons and liver formation [Maina et al., 1997]. These results suggest that different combinations of downstream pathways are involved in the development of different organs and tissues, and seem to be utilized differentially in HGF/SF-induced invasion and branching morphogenesis. Interestingly, targeted disruption of Grb2 in mice shows a placental defect [Saxton et al., 2001], similar to that of HGF/SF or Met null mice. Would it be possible to rescue the phenotypes observed with one Met mutant by complementation with a different Met mutant molecule? For example, by intercrossing different lines of Met mutant mice such as Src optimal Met mutant mice together with Met mutant mice optimized for PI3K or Grb2.

INTRACELLULAR SIGNALING IN BRANCHING MORPHOGENESIS AND INVASION

Following Met activation, diverse intracellular signaling pathways are activated to mediate HGF/SF induced invasion and branching morphogenesis (Fig. 1). PI3K and Ras/MAPK cascades are two major pathways that have been intensively studied and well characterized. PI3K pathway is coupled to Met through the interaction of its p85 subunit with the multi-docking sites of Met, while Ras/MAPK pathway is bridged by the adaptor protein Grb2 which links Met to SOS, a Ras guanine nucleotide exchange factor [Ponzetto et al., 1994]. HGF/SF induces scattering and branching tubulogenesis of Madin-Darby canine kidney (MDCK) cells which is the original in vitro system for assaying HGF/SF activities [Stoker et al., 1987; Montesano et al., 1991]. Treatment of MDCK cells with PI3K inhibitor LY294002 or Wortmannin abolishes HGF/SF induced scattering, suggesting that PI3K pathway is essential for cell motility [Royal et al., 1997]. When treated with PD98059, an inhibitor for MAPK pathway, MDCK cells also show loss of motility and fail to form branching tubule structures in response to HGF/SF [Khwaja et al., 1998]. HGF/SF, through activated ERK, also induces the phosphorylation and activation of paxillin and focal adhesion kinase (FAK) [Liu et al., 2002]. This activation is abrogated with U0126, (another ERK inhibitor) which also inhibits HGF/SF-induced cell spreading and adhesion [Liu et al., 2002]. It has been shown that Ras is required for epithelial adhesion junction dissembly induced by HGF/SF through activation of both PI3K and MAPK [Potempa and Ridley, 1998]. Collectively, these studies provide strong evidence that both PI3K and Ras/MAPK pathways are required for HGF/SF-induced invasion and branching morphogenesis.

In addition to PI3K and Ras/MAPK pathways, activated Met also recruits Gab1, Src and Stat3 to its multi-docking sites. Gab1, the large scaffolding molecule that is phosphorylated in association with activated Met, is also responsible for HGF/SF-Met-induced scattering and branching morphogenesis of epithelial cells [Weidner et al., 1996]. Gab1 brings a number

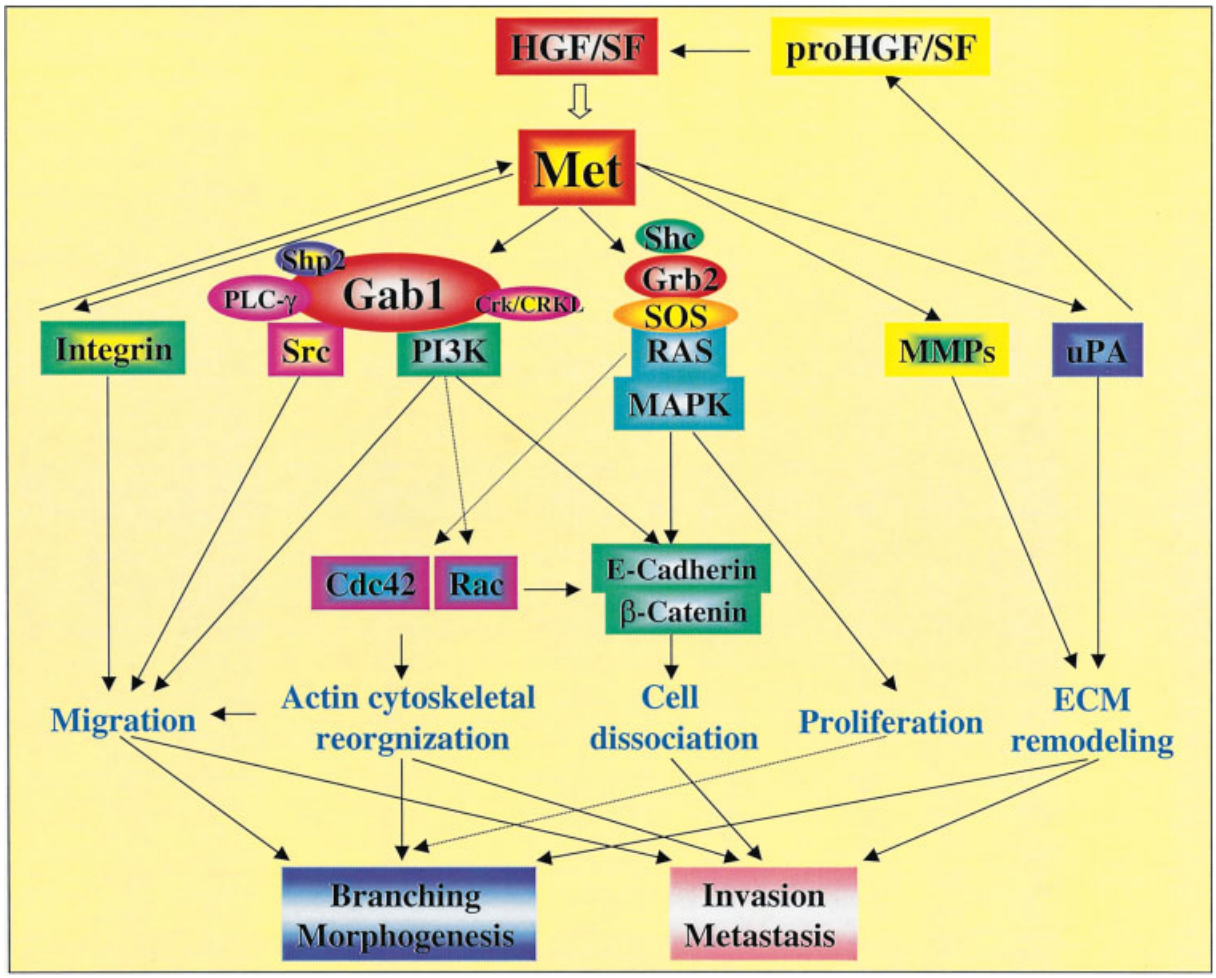

Fig. 1. Schematic representation of HGF/SF-Met signaling cascades in the control of branching morphogenesis and invasion. Upon HGF/SF binding, Met can activate a series of intracellular signaling pathways such as Ras/MAPK, PI3K, and Src through the two major adaptor molecules Grb2 and Gab1, which induces the biological events including proliferation, cell dissociation, and migration. Met can activate Cdc42 and Rac through a manner without direct interaction, which lead to the actin cytoskeletal reorgnization, an event crucial for branching morphogenesis and invasion. Interaction of Met with integrin in a ligand-dependent or independent manner is also critical for Met-mediated invasive growth. Except the enzymatic changes of downstream signaling molecules, HGF/SF-Met signaling also increases the activities of MMPs and uPA by up-regulating these gene expressions, which contributes to the ECM remodeling as well as activation of HGF/SF.

of substrates to the multi-docking sites for Met phosphorylation and activation, including PLC-γ, Shc, Shp2, and CRKL as well as Grb2 and PI3K. Uncoupling of PI3K, PLC-γ or Shp2 from Gab1 impairs Met-mediated branching morphogenesis [for review see Furge et al., 2000]. The biological importance of Gab1 downstream of HGF/SF-Met signaling has been emphasized from germline knockout studies. Embryos homozygously deleted for Gab1 display phenotypes similar to embryos nullyzygous for HGF/SF and c-Met, including placental defects in the labyrinth layer and defects in the muscle group originating from a deficit in migratory precursor cells [Sachs et al., 2000]. These results suggest that Gab1 together with Grb2 are two essential adaptors for HGF/SF-Met signaling. Disruption in either one will impair the signaling transduction required for HGF/SF-induced placental morphogenesis.

The non-receptor tyrosine kinase Src is a major mediator for Met signaling and is also required for HGF/SF induced motility as measured in mammary carcinoma cells [Rahimi et al., 1998]. Although Stat3 is also recruited to the multi-docking sites of Met and activated by

HGF/SF, we found that Stat3 is required for anchorage independent growth and HGF/SF-induced branching morphogenesis [Boccaccio et al., 1998; Zhang et al., 2002]. Recently, SH2 domain—containing inositol 5-phosphastase 1 (SHIP-1) was identified to be a new binding partner for Met via Y^{1356} at the multi-docking sites [Stefan et al., 2001]. Overexpression of SHIP-1 enhances HGF/SF-induced branching tubulogenesis, while mutant SHIP-1 impairs this process, suggesting that SHIP-1 is another critical factor for HGF/SF-Met mediated branching morphogenesis.

During invasion or branching morphogenesis, the driving forces for cell motility are derived from the cytoskeletal reorganization of actin which is controlled by Cdc42, Rac and Rho small GTPases [for review see Ridley, 2001]. Cdc42 promotes filapodia and microspikes formation while Rac induces lamellipodia and membrane ruffling. HGF/SF can induce the activation of Cdc42, Rac and Rho, concomitant with the formation of filapodia, lamellipodia and membrane ruffling [Royal et al., 2000]. Several effectors for Cdc42 and/or Rac have also been found to be involved in HGF/SF-induced cell–cell dissociation and migration, such as Cdc42/Rac-regulated p21-activated kinase (PAK) [Royal et al., 2000] and neural Wiskott-Aldrich syndrome protein (N-WASP) [Yamaguchi et al., 2002]. However, it is not quite clear how HGF/SF activates the Rho GTPase family. For example does crosstalk occur between Ras and Rho pathways. It is not clear whether HGF/SF induces the activation of Rho family indirectly through Ras pathway or by recruiting unidentified effector(s) of Rho family to Met or its adaptor proteins.

ECM AND ADHESION MOLECULES IN BRANCHING MORPHOGENESIS AND INVASION

Invasion and branching morphogenesis require not only cell–cell communications, but also communication between the cell and the ECM. ECM plays a significant role in HGF/SF-induced biological activities: ECM not only provides the milieu for cells to move but is a reservoir of factors required for the cleavage and activation of HGF/SF (such as uPA and tPA). Integrins are receptors for many ECM components, such as fibronectin, vitronectin, laminin and collagen, and are key factors in the coordination of cell–cell and cell–ECM adhesion [Giancotti and Ruoslahti, 1999]. Binding of ECM components to integrin triggers integrin clustering and association with the cytoskeleton, leading to the attachment of cells to ECM. HGF/SF can trigger the clustering of integrins concomitant with promotion of cell invasion [Trusolino et al., 2000], suggesting that integrins may play a role in HGF/SF-Met mediated invasive growth. Previously, it has been shown that $\alpha 2\beta 1$ integrin is necessary for HGF/SF-induced branching morphogenesis of MDCK cells [Saelman et al., 1995]. Recently, another integrin, $\alpha 6\beta 4$, has been shown to function as a signaling adaptor in HGF/SF-Met mediated invasion [Trusolino et al., 2001]. Interestingly, in a Met transgenic animal model, Wang et al. showed that cell attachment could induce and sustain the activation of Met receptor leading to hepatocellular carcinomas in the absence of HGF/SF ligand [Wang et al., 2001]. It is not clear what leads to the activation of Met, but one strong possibility might be through integrin mediated cell attachment. Ligand-independent activation of growth factor receptors (including Met), by cell adhesion through integrin has been widely observed [for review see Schwartz and Ginsberg, 2002].

HGF/SF also induces disruption of cell–cell adhesion junction by dispersal of E-cadherin and β-catenin from the intercellular junction. This action can be inhibited by the MAPK inhibitor PD98059, the PI3K inhibitor LY294002 or by a dominant negative Rac1 molecule [Potempa and Ridley, 1998]. Introduction of either mutant cadherin or mutant β-catenin into MDCK cells impairs branching tubulogenesis induced by HGF/SF [Pollack et al., 1997; Troxell et al., 2001]. These results suggest that E-cadherin-β-catenin complex-mediated cell adhesion is required for HGF/SF-induced branching tubulogenesis as well as invasion. β-catenin is a key player in the Wnt signaling pathway. It will be interesting to see whether HGF/SF pathway somehow interplays with Wnt pathway during branching or invasion [for review see Vande Woude et al., 1997].

During invasion and branching morphogenesis, the ECM in the path of the invading cell surrounding the tip of the branch must be degraded and remodeled. Many proteases such as uPA and matrix metalloproteases (MMPs) are involved in the process of ECM degradation and remodeling. HGF/SF-induced branching

TABLE I. Summary of Signaling Molecules Required for the HGF/SF-Met Mediated Branching, Motility or Invasion*

Signaling molecules	HGF/SF-induced activity (in vitro)			HGF/SF-Met activity in development (in vivo)	References[a]
	Cell type tested	Branching	Motility or invasion		
Grb2	MDCK		+	Essential for placental development	Furge et al., 2000; Saxton et al., 2001[a]
Shc	MDCK		+		Furge et al., 2000[a]
Ras-MAPK	MDCK	+	+		Royal et al., 1997; Khwaja et al., 1998
Gab1	MDCK	+	+	Essential for the development of placenta and migratory skeletal muscle	Weidner et al., 1996; Sachs et al., 2000
PI3K	MDCK	+	+	Sufficient for Met-mediated sensory neuron branching, but not for placenta formation or myoblast migration	Royal et al., 1997; Maina et al., 2001; Khwaja et al., 1998
Shp2	MDCK	+			Schaeper et al., 2000
PLC-γ	MDCK	+			Furge et al., 2000[a]
CrK/CRKL	MDCK	+	+		Furge et al., 2000; Lamorte et al., 2002[a]
SHIP-1	MDCK	+	+		Stefan et al., 2001
SRC	SP1		+	Sufficient for Met-mediated placental development, but not for myoblast migration or sensory neuron branching	Rahimi et al., 1998; Maina et al., 2001
Stat3	MDCK, SK-LMS-1	?	?		Boccaccio et al., 1998; Zhang et al., 2002
Cdc42, Rac, PAK	MDCK		+		Sander et al., 1998; Royal et al., 2000
N-WASP	MDCK	+	+		Yamaguchi et al., 2002
Integrin	MDCK, MDA-MB-435	+	+		Saelman et al., 1995; Trusolino et al., 2001
Cadherin	MDCK	+			Troxell et al., 2001
β-catenin	MDCK	+			Pollack et al., 1997

*Summary is derived from the referred studies. Surprisingly, most of the in vitro evidence is derived from MDCK cell and not other cell lines.
[a]Due to limitation of number of references allowed, previous reviews were favored for citations.

and tubulogenesis of epithelial cells can be modulated by the different components of ECM [Santos and Nigam, 1993]. HGF/SF-Met signaling increases the expression of uPA and its receptor, thereby enhancing the activity of uPA [Pepper et al., 1992; Jeffers et al., 1996]. MMP expression is also induced by HGF/SF treatment [Kermorgant et al., 2001] and there is direct evidence showing that MMP activity is necessary for branching morphogenesis of mammary epithelial cells induced by different growth factors including HGF/SF [Simian et al., 2001]. Increasing uPA and MMP activity is correlated with enhanced invasiveness induced by HGF/SF [Jeffers et al., 1996; Kermorgant et al., 2001], suggesting that HGF/SF-Met can integrate these processes for branching morphogenesis and invasion through its own signaling.

PERSPECTIVE

Both invasion and branching morphogenesis are complicated cellular events and require simultaneous and/or sequential actions of many factors and pathways. Although many pathways have been shown to be involved in the process of invasion and branching morphogenesis (Fig. 1), the mechanism of how HGF/SF via c-Met induces these complicated cellular processes is not well understood. Surprisingly, most of the in vitro evidence as summarized in Table I is derived from MDCK cells. How are these factors and pathways activated and inactivated temporally and spatially in response to HGF/SF-Met signaling? Many of the pathways are actually shared and activated by many different growth factors or cytokines, however, the need for specificity of response is not clear. Whether differences in duration of signal, and/or different combinations of factors and pathways are sufficient to explain the specificity of response or whether there exists unidentified factor(s) or pathway(s) that can explain the differences remains to be seen. What kind of de novo gene expression might be required for the regulation of HGF/SF-Met mediated invasion and branching morphogenesis, either in a positive or negative fashion? Fully understanding the mechanism of HGF/SF-Met induced cellular events may provide a guidebook to exploit new drugs or compounds for therapeutic intervention on invasion and metastasis, and to have a clear view on how aberration in this pathway may lead to the defects of individual organ or tissue during development, which may benefit clinical organ or tissue regeneration after damage.

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