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The Met RTK: from Tubes to Tumorigenesis

Morag Park,

Molecular Oncology Group, McGill University Hospital Centre, Montreal

Abstract

The receptor for hepatocyte growth factor/scatter factor (HGF/SF), Met, controls a program of invasive epithelial growth through the co-ordination of cell proliferation and survival, cell migration and epithelial morphogenesis. This process is important during embryogenesis and for organ regeneration in the adult. However, when deregulated the HGF/SF-Met signalling axis contributes to tumorigenesis and metastasis.

Discovery of Met and HGF/SF

Hepatocyte growth factor, the ligand for the Met receptor tyrosine kinase, was originally identified as a mitogen for hepatocytes in culture (Nakamura et al., 1989; Zarnegar and Michalopoulos, 1989). HGF is identical to scatter factor, a fibroblast-derived factor that promotes dispersal of sheets of epithelial cells (Stoker et al., 1987) as well as branching tubulogenesis of epithelia grown in three dimensional cultures (Montesano et al., 1991a). HGF/SF is thus a unique growth factor that elicits multiple cellular responses including mitogenesis, cell motility and morphogenesis (reviewed by Comoglio and Boccaccio, 2001; Birchmeier et al., 2003). HGF/SF is produced primarily by mesenchymal cells and is secreted as an inactive precursor (pro-HGF/SF) which is activated by proteolytic cleavage into disulfide-linked a and b chains (Nakamura, 1991).

The high affinity receptor for HGF/SF is the Met receptor tyrosine kinase (Bottaro et al., 1991). Met was first identified as the product of a human oncogene, generated following a chromosomal rearrangement, where a protein dimerization motif (Tpr) is fused to the cytoplasmic kinase domain of Met (Cooper et al., 1984; Park et al., 1986) (Fig.

1). Cloning of the Met gene identified it as a transmembrane receptor tyrosine kinase (Park et al., 1987). The Tpr-Met fusion protein is oncogenic, and is constitutively dimerized and activated in the absence of HGF/SF (Rodrigues and Park, 1993). Tpr-Met now acts as a prototype for a large family of receptor tyrosine kinase (RTK) oncogenes that are activated following chromosomal rearrangements in human tumors (Rodrigues and Park, 1994b; Lamorte and Park, 2001).

Structure of Met and HGF/SF

Met is synthesized as a single chain precursor of 170 kDa that is glycosylated, then cleaved at a furin site, as it matures on the cell surface, to generate a disulfide-linked heterodimer of 190 kDa

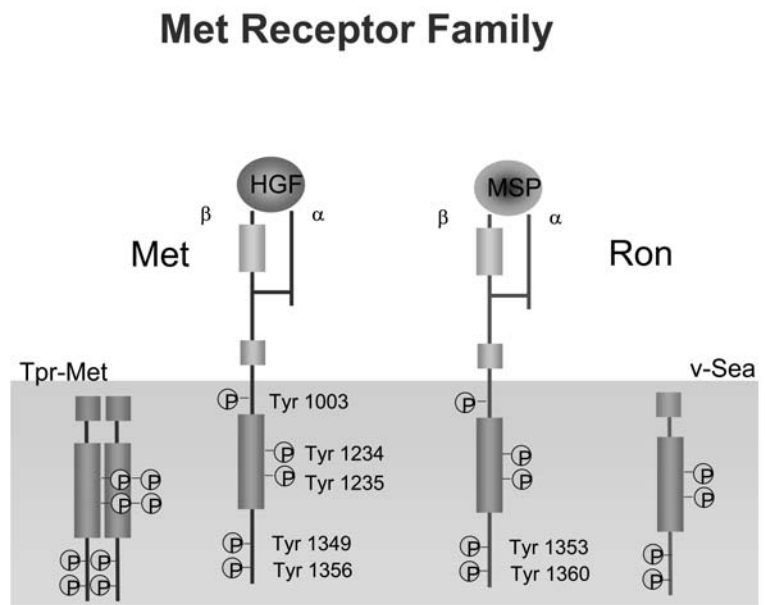


Figure 1. The Met receptor tyrosine kinase family contains the Met and Ron RTKs. Met was first identified as an activated oncogene, Tpr-Met. V-Sea is the avian oncogenic homologue of Ron

(Giordano et al., 1989; Komada et al., 1993). This consists of a 50 kDa extracellular α -chain and 140 kDa membrane spanning β -chain (Fig. 1). The latter contains the juxtamembrane and kinase domains as well as the C-terminal tail, which is essential for downstream signalling. Based on shared structural homology, Met is classified as the prototype member of a RTK subfamily that also contains the Ron RTK (Ronsin et al., 1993) (Fig. 1). In addition, the ligand for Ron, macrophage stimulating protein (MSP), is highly homologous to HGF (Gaudino et al., 1994). HGF and MSP contain a domain structure typical of the proteinases of the plasminogen family, and constitute a family with related biological activities, termed plasminogen-related growth factors (Donate et al., 1994). The extracellular portions of Met and Ron, contain a region of homology to the SEMA domain of the semaphorin axon guidance proteins (Maestrini et al., 1996; Artigiani et al., 1999). This region contains the α -chain, and 212 residues of the β -chain, that together are predicted to fold into a β -propellor structure (Gherardi et al., 2003). β -propellor structures are found in other proteins and are thought to be involved in protein-protein interaction (Xiong et al., 2002). In Met, the integrity of this domain is required for HGF/SF binding, as well as receptor dimerization (Gherardi et al., 2003; Kong-Beltran et al., 2004).

Functions of HGF/SF and Met

HGF/SF and Met are expressed in many tissues in the adult. In these tissues, HGF/SF is produced by mesenchymal cells and activates its receptor Met, expressed in epithelial and endothelial cells through a paracrine mode of action. HGF/SF is a potent mitogen for primary hepatocytes and renal tubule cells (Zarnegar and Michalopoulos, 1989), stimulates epithelial cell dissociation and invasion (Stoker et al., 1987), and acts as an initiating signal for an intrinsic cellular morphogenic program of kidney, breast and lung epithelium grown in matrix culture (Montesano et al., 1991b; Weidner et al., 1993; Sachs et al., 1996). In the adult, HGF/SF and Met are thought to be involved in a general repair of tissue damage. Elevated levels of HGF/SF and Met are observed in both injured tissues following kidney, liver or heart injury, as well

as in the circulating plasma (Michalopoulos and DeFrances, 1997; Nakamura et al., 2000; Matsumoto and Nakamura, 2001). HGF/SF has cytoprotective activity *in vivo* and protects against various types of tissue injury (Roos et al., 1995; Jin et al., 2003). HGF/SF is also a potent angiogenic factor (Grant et al., 1993) through induction of VEGF, a positive regulator of angiogenesis, as well inhibition of thrombospondin, a negative regulator of angiogenesis (Zhang et al., 2003; Saucier et al., 2004).

HGF/SF and Met are essential during embryogenesis and mice null for either gene die in utero, with reduced proliferation and survival of placental trophoblasts as well as hepatocytes (Schmidt et al., 1995; Uehara et al., 1995). This is consistent with HGF/SF acting as a potent mitogen for hepatocytes and the important role of Met in liver regeneration (Borowiak et al., 2004; Huh et al., 2004). In addition, these studies have demonstrated a role for Met and HGF/SF in the development and innervation of skeletal muscle, and directing the growth of axonal cones (Schmidt et al., 1995; Uehara et al., 1995; Yang and Park, 1995; Ebens et al., 1996; Maina et al., 1997). The phenotypes of the Met and HGF/SF null mice are identical, indicating that during embryogenesis Met is the only receptor for HGF/SF and vice versa (Schmidt et al., 1995; Uehara et al., 1995; Birchmeier and Gherardi, 1998).

Met signal transduction

While signaling pathways downstream from RTKs involved in a mitogenic response had been characterized in detail, until recently, little was known about the signalling pathways involved in the complex program of invasive growth regulated by the Met receptor. Epithelial cells, and in particular, the Madin-Darby canine kidney (MDCK) cell line, respond to HGF/SF and Met signals by scattering in two dimensional cultures, and in the formation of branching tubules in three dimensional cultures (Fig. 2), and have been a cell line of choice to dissect the signals involved in these processes. HGF/SF-induced dispersal of epithelial colonies occurs in a stepwise transition, where HGF/SF first promotes the breakdown of cell-cell junctions, and

induces changes in epithelial morphology to cells of a more fibroblastic cell shape, with increased motility and invasiveness (Royal and Park, 1995)(Fig. 2). When seeded in a collagen matrix, MDCK cells form hollow cysts of polarized epithelial cells (Fournier et al., 1996). Treatment of MDCK cells with HGF/SF induces the formation of branching tubules (Weidner et al., 1993). Tubular branching is a complex morphogenic process that requires tight co-ordination of cell growth, cell polarity, movement and invasion (reviewed in Pollack et al., 1998).

The use of receptor chimeras demonstrated that the Met cytoplasmic domain is sufficient to mediate all of the pleiotropic biological responses attributed to HGF in epithelial cells, and that these events require Met protein tyrosine kinase activity (Zhu et al., 1994b). Phosphorylated tyrosine residues in the non-catalytic cytoplasmic domains of RTKs act as specific binding sites for Src homology 2 (SH2) and phosphotyrosine binding (PTB) domain-containing proteins, and these in turn transduce intracellular signals (reviewed in Pawson and Scott, 1997).

Upon stimulation with HGF/SF, the Met receptor cytoplasmic domain becomes highly phosphorylated on tyrosine residues (Zhu et al., 1994b). The phosphorylation of two tyrosine residues within the activation loop of the kinase domain is required for the intrinsic kinase activity of Met (Rodrigues and Park, 1994a). Two tyrosine residues within the carboxyl terminus (Y1349 and Y1356) are crucial for cell scatter and branching morphogenesis in Madin-Darby canine kidney (MDCK) epithelial cells (Ponzetto et al., 1994; Zhu et al., 1994a; Fixman et al., 1995). Phosphorylation of these tyrosine residues generates a multisubstrate docking site that is highly conserved between other members of the Met RTK gene family, v-Sea and Ron.

When phosphorylated, Y1356 provides a direct binding site for the Grb2 and Shc adapter proteins, as well as the p85 subunit of PI3kinase (Fig. 3). Shc and Grb2 can couple Met to the Ras-MAPK pathway. In addition, Grb2 acts as an adapter to indirectly recruit multiple proteins to Met. These

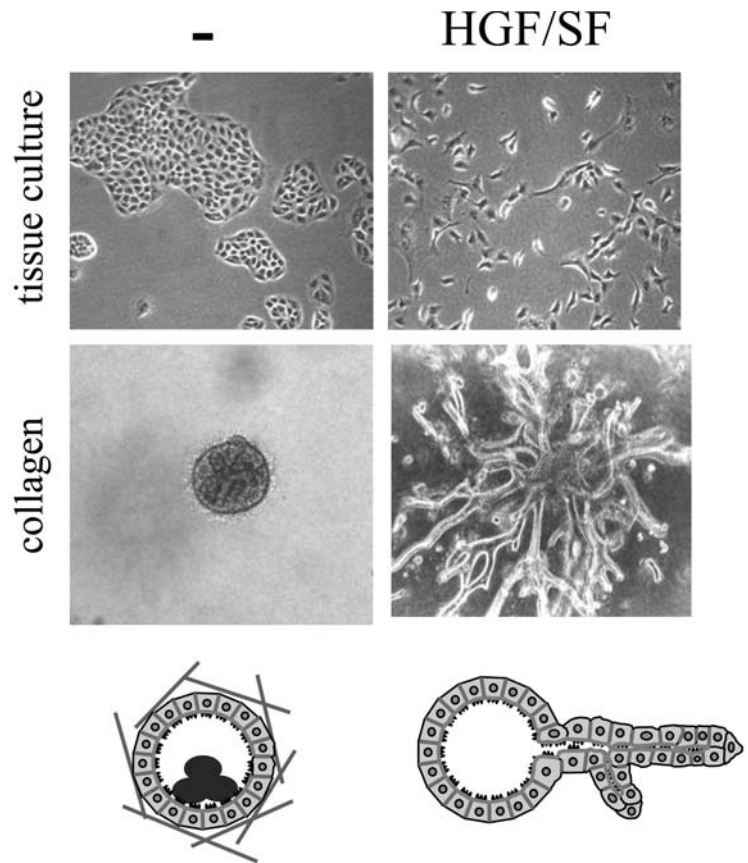


Figure 2. Hepatocyte growth factor scatter factor (HGF/SF) stimulates cellular scattering and branching morphogenesis. HGF/SF stimulates the scattering of colonies of MDCK cells grown on plastic dishes, but stimulates the reorganization of these cells from a hollow cyst into branching tubules when grown in three-dimensional collagen gels.

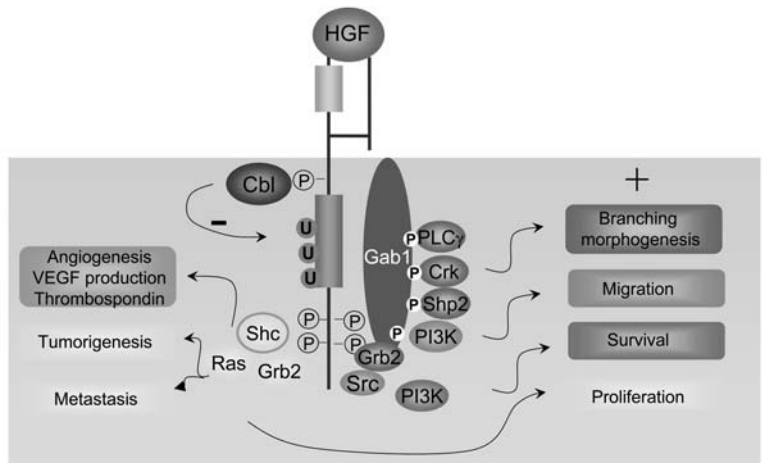


Figure 3. Substrate binding sites and recruitment of proteins to the Met receptor. Twin tyrosines in the carboxyterminal tail of Met (Y1349 and I356) act as a multi-substrate binding site conserved in Met family members. Y1356 serves to recruit the adaptor proteins Grb2 and Shc. Grb2 in turn indirectly recruits Gab1 and Cbl to Met. Once phosphorylated, Gab1 recruits the SHP-2 tyrosine phosphatase, Crk adaptor protein, phosphatidylinositol-3'-kinase (PI3'kinase) and phospholipase C_γ.

include the Gab1 docking protein as well as the cCbl ubiquitin ligase. Gab1 was initially identified in a library screen as a Grb2 binding protein (Holgado-Madruga et al., 1996) and belongs to a family of docking proteins, including closely related proteins Gab2 and Daughter of Sevenless (DOS), and the more remotely related, Insulin Receptor Substrate-1 (IRS-1), IRS-2, IRS-3, Downstream of Kinases (Dok), and FGF receptor substrate 2 (FRS2) (reviewed in Liu and Rohrschneider, 2002). These proteins lack enzymatic activities. Following activation of tyrosine kinase, and cytokine receptors, they become phosphorylated on tyrosine residues, providing binding sites for multiple proteins involved in signal transduction. In this manner they act to potentiate and diversify the signals downstream from receptors by virtue of their ability to assemble multiprotein complexes.

Gab1 and tubulogenesis

In epithelial cells, Gab1 is the major substrate for the Met receptor tyrosine kinase (Nguyen et al., 1997), and from genetic (Sachs et al., 2000) and cell biological studies (Maroun et al., 1999a; Maroun et al., 1999b; Maroun et al., 2000; Maroun et al., 2003), Gab1 is crucial for many of the biological responses downstream from Met. Although Gab1 is recruited to many RTKs indirectly through Grb2, its interaction with the Met receptor is unique and involves both indirect as well as direct recruitment of Gab1 (Lock et al., 2000; Lock et al., 2002). The direct interaction of Gab1 with Met requires 13 amino acids within the Gab1 Met binding domain that interact directly with Y1349 of the multisubstrate binding site on the Met C-terminus (Schaeper et al., 2000). This sequence does not resemble classical SH2 or PTB domains and is not conserved in other members of the Gab family. Instead it associates with the Met receptor as a peptide motif that requires structural integrity and amino acids within the Met kinase domain for binding to pY1349 (Lock et al., 2003). This defines a unique interaction between a RTK and its substrate. It allows a direct and robust association between Gab1 and Met that results in prolonged phosphorylation of Gab1 in response to HGF. Prolonged signalling downstream from Gab1

is required for the morphogenic response, whereas other receptors, such as the epidermal growth factor receptor (EGFR), that recruit Gab1 indirectly through Grb2, are unable to induce a morphogenic response in MDCK cells (Maroun et al., 1999a).

Upon tyrosine phosphorylation, Gab1 interacts with multiple proteins involved in signal transduction through their SH2 domains. These include the tyrosine phosphatase, SHP-2, the p85 subunit of PI3'K, PLC γ , as well as the Crk adaptor protein (Holgado-Madruga et al., 1996; Garcia-Guzman et al., 1999; Maroun et al., 1999a; Gual et al., 2000; Maroun et al., 2000; Schaeper et al., 2000; Ma et al., 2003; Maeda et al., 2004). The association of Gab1 with the SHP-2 phosphatase or the Crk adapter protein, as well as an intact Gab1 PH domain, is required for the ability of Gab1 to promote the morphogenic program of MDCK epithelial cells downstream from the Met receptor (Fig. 3) (Maroun et al., 1999a; Maroun et al., 2000; Lamorte et al., 2002b). The PH domain of Gab1 binds to phosphatidylinositol-3,4,5-trisphosphate (PIP3), which is essential for subcellular localization of Gab1 and efficient branching tubulogenesis downstream from the Met receptor (Maroun et al., 1999a; Maroun et al., 1999b). SHP-2 contains two tandem SH2 domains followed by a phosphatase domain. Both tyrosine phosphorylation of SHP-2, and binding of its SH2 domains to tyrosine-phosphorylated peptides, enhance its catalytic activity (Neel et al., 2003), possibly through the release of negative regulatory constraints on the phosphatase domain mediated by the SH2 domains of SHP-2 (Barford and Neel, 1998). SHP-2 enhances MAPK activation downstream from Met, indicating a positive function in Met signalling (Fig. 3) (Maroun et al., 2000; Schaeper et al., 2000). The importance for Gab1 downstream from Met signalling has been supported from knock-out studies. Embryos nullizygous for Gab1 display all of the defects observed in Met or HGF/SF null embryos (Itoh et al., 2000; Sachs et al., 2000).

The formation of tubules from polarized epithelia grown in three dimensional organ cultures is a complex cellular response that requires all known Met signals (reviewed in Rosario and Birchmeier,

2003; Zegers et al., 2003). From the use of inhibitors, PI3K and ERK/MAPK and Src pathways are required for the disassembly of adherens junctions cell spreading and cell motility (Royal and Park, 1995; Potempa and Ridley, 1998; Rahimi et al., 1998). The sustained ERK kinase cascade, mediated by Gab1-SHP-2 interactions, is required for the remodelling of adherens junctions and cell proliferation during the morphogenic response (Maroun et al., 2000; Schaeper et al., 2000). Met also activates pathways that modulate the actin cytoskeleton, Src as well as Rho, Rac and PAK that control cytoskeleton rearrangement, cell adhesion and migration (Rahimi et al., 1998; Royal et al., 2000). Some of these are mediated through Gab1-Crk interactions, whereby Crk can couple Gab1 to Rap1 and Rac (Lamorte et al., 2002a). A functional Crk protein is also required for breakdown of cell-cell junctions and cell dispersal, as well as branching morphogenesis in response to HGF/SF (Lamorte et al., 2002b). Cell survival is primarily controlled through PI3K dependent activation of Akt and downstream pathways (reviewed in Birchmeier et al., 2003).

Crosstalk with other signalling pathways

In addition to HGF/SF, Met is the major host receptor for the InlB protein of *L. monocytogenes* (Shen et al., 2000). Entry of *L. monocytogenes* into epithelial cells, endothelial cells and hepatocytes is considered to play an important role in its pathogenesis. InlB/Met interactions promote entry of *L. monocytogenes* in cells that are normally non-phagocytic. This occurs through the activation of Met-dependent signalling pathways. These promote remodelling of the actin cytoskeleton required for phagocytic entry of the bacterium and involves Gab1 and recruited PI3K and Crk (Sun et al., 2005).

Met can also interact with several cell surface proteins that influence its activity and cooperate with Met to elicit a biological response. These include $\beta 4$ integrin (Trusolino et al., 2001), the hyaluronan receptor CD44 (Orian-Rousseau et al., 2002), the Fas receptor (Wang et al., 2002), semaphorin (Giordano et al., 2002) and ezrin (Crepaldi et al.,

1997). These complexes may act to localize Met signals to specific membrane microenvironments, as well as establishing complexes whose signals act in a synergistic manner. For example, in a variety of carcinoma cells, HGF/SF-induced cell invasion is coupled with association between Met and $\alpha 6\beta 4$ integrin, where activation of Met induces tyrosine phosphorylation of $\beta 4$ integrin and enhanced integrin signalling (Trusolino et al., 2001). Similar complexes between Met and semaphorin, or Met and CD44, have been associated with invasive growth (Orian-Rousseau et al., 2002). In addition, Met signals co-operate with the HER-2 RTK to promote loss of epithelial polarity and organization, and enhanced cell invasion, in three dimensional epithelial cell cultures, although in this case no physical interaction between Met and HER-2 was observed (Khoury et al., 2005).

HGF/SF and Met in tumorigenesis

The expression pattern of Met and HGF/SF, promotes crosstalk between the epithelial and stromal compartments required for normal physiological processes (Thiery, 2003). Under normal conditions HGF/SF and Met crosstalk is tightly regulated (Taub, 2004). Deregulation of the Met and HGF/SF signalling axis occurs in many human tumors, through co-expression of HGF/SF and Met, through receptor amplification and through point mutations in the juxtamembrane domain as well as the kinase domain of Met (reviewed in Birchmeier et al., 2003 and www.vai.org/HgfSf-METandcancer). Overexpression of HGF/SF alone in mammary stroma of mice is sufficient to promote loss of organization of mammary epithelium corresponding to hyperplasia (Kuperwasser et al., 2004). Mutations within the tyrosine kinase domain of Met have been found in both sporadic and hereditary forms of human papillary renal cancer (Schmidt et al., 1997), whereas mutations in the juxtamembrane domain of Met are found predominantly in human gastric and lung cancers (Lee et al., 2000; Ma et al., 2003). Under experimental conditions, Met receptors with these mutations are transforming in fibroblasts (Jeffers et al., 1997a; Jeffers et al., 1998; Lee et al., 2000; Ma et al., 2003). Some are tumorigenic in transgenic ani-

mals, (Jeffers et al., 1998) and when substituted in the germline, induce multiple tumor types (Graveel et al., 2004).

The ability of Met to drive cells to invade and form metastases has been validated in model systems, involving transfected cells as well as transgenic animals (Rong et al., 1994; Jeffers et al., 1996; Saucier et al., 2002). Pathways driving metastatic spread are considered to represent the deregulated activation of those corresponding to the invasive growth regulated by Met during embryogenesis and under physiological conditions. Structure-function studies have identified the requirement of Grb2 and Shc dependent pathways for cell transformation, tumorigenesis and metastatic spread (Fixman et al., 1996; Saucier et al., 2002). Grb2 recruitment not only activates the Ras-MAPK pathway, but also recruits the docking protein Gab1. These studies have revealed a role for the Shc signalling pathway, independent of Grb2, for tumorigenesis through the induction of VEGF. This is required for an angiogenic response downstream from Met as well as the HER2 RTK, a step essential for tumor growth (Saucier et al., 2004).

Cbl and Met receptor endocytosis and degradation.

In addition to the enhanced Met signaling observed with oncogenic Met receptor mutants, recent studies have demonstrated that loss of negative regulation also contributes to oncogenic activation of Met (Peschard et al., 2001a), and may represent a common mechanism that contributes to oncogenic activation of other RTKs in human cancer (Peschard and Park, 2003). The rapid removal of growth factor receptors from the cell surface, and subsequent targeting to lysosomal degradative compartments, provides a downregulation mechanism important for preventing sustained stimulation, which could potentially lead to cellular transformation (Fig. 5) (reviewed in Marmor and Yarden, 2004). Many of these interactions are dependent on RTK activation and ubiquitination (Katzmann et al., 2001; Buchberger, 2002; Davies et al., 2004) which involve the Cbl family of ubiquitin ligases (Joazeiro et al., 1999;

Keane et al., 1999; Yokouchi et al., 1999; Thien and Langdon, 2001).

The recruitment of the Cbl family of ubiquitin-protein ligases is required for ligand-induced degradation of many RTKs, among them the EGFR, the platelet-derived growth factor receptor (PDGFR), the colony-stimulating factor-1 receptor (CSF-1R) (reviewed by Thien and Langdon, 2001) and the Met receptor (Peschard et al., 2001b; Peschard et al., 2004). Growing evidence indicates that ubiquitination of RTKs is critical for their lysosomal degradation through their ubiquitin-dependent protein sorting, which retains RTKs in late endosomes and subsequently targets these receptors to intraluminal vesicles of MVBs and lysosomal degradation (Urbe et al., 2000; Raiborg et al., 2002; Duan et al., 2003; Jiang et al., 2003; Yamasaki et al., 2003). RTKs including Met are multi-monoubiquitinated rather than being polyubiquitinated (Haglund et al., 2003; Mosesson et al., 2003; Carter et al., 2004). The multi-monoubiquitinated RTKs may be selectively recognized by proteins of the endocytic pathway that contain a ubiquitin-interacting motif (UIM), such as Epsin, Eps15 and Hrs (Hepatocyte Growth Factor regulated tyrosine kinase substrate) (Hofmann and Falquet, 2001) among others.

Stimulation of the Met receptor with HGF/SF induces tyrosine phosphorylation of the receptor, and stimulation with high levels of HGF/SF leads to detectable Met receptor ubiquitination and enhanced degradation (Jeffers et al., 1997b; Kamei et al., 1999; Shen et al., 2000). The juxtamembrane region of Met contains an additional docking site at Y1003, which acts as a negative regulator of Met biological activity, and is absent in the Tpr-Met oncogene (Fig. 1). Although c-Cbl can be recruited to Met indirectly through the Grb2 adapter protein (Fig. 2), phosphorylation of Y1003 provides a direct docking site for the SH2-like TKB domain of the c-Cbl ubiquitin ligase, and is required for ubiquitination and ligand-dependent degradation of the Met receptor (Peschard et al., 2001b). Met receptor mutants uncoupled from Cbl dependent ubiquitination are transforming and tumorigenic, through enhanced stability of Met

and sustained signalling of downstream pathways (Peschard et al., 2001b).

Although a consensus for c-Cbl TKB domain binding has been established (D/NxpYxxD/EF), this motif is not present in Met, and instead, a DpYR motif, including Y1003, is required for the direct recruitment of the c-Cbl TKB domain and for ubiquitination of the Met receptor (Peschard et al., 2004). The DpYR motif is conserved within Met family members, Met, Ron and Sea, as well as in Met orthologues in puffer fish, suggesting a conserved function for this motif in Cbl recruitment and negative regulation of the Met receptor family (Penengo et al., 2003).

Loss of Cbl ubiquitylation in oncogenic RTKs

The observation that the specific uncoupling of the Met receptor from ubiquitination is associated

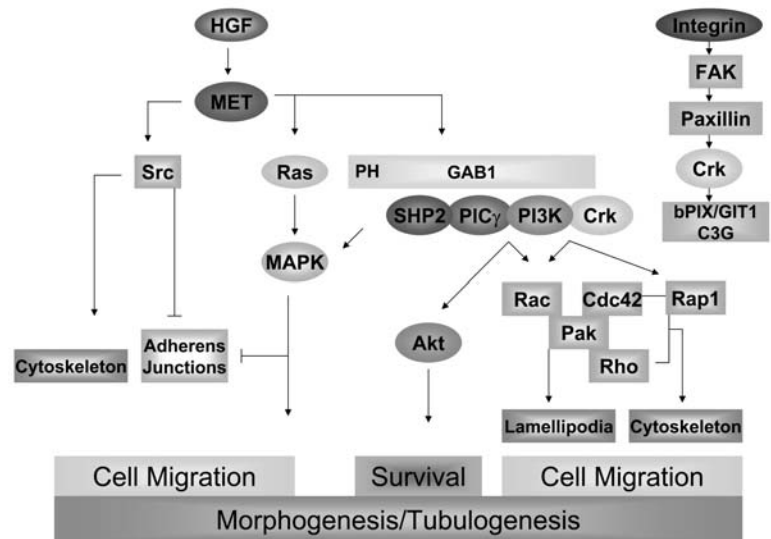


Figure 4. Signalling by the Met receptor tyrosine kinase during tubulogenesis. Activation of the Met receptor results in the recruitment of numerous signalling proteins to the receptor. Regulation of cellular proliferation, adhesion, cytoskeletal reorganization and cell survival are required to co-ordinate the reorganization of cysts of polarized epithelial cells into polarized tubular structures.

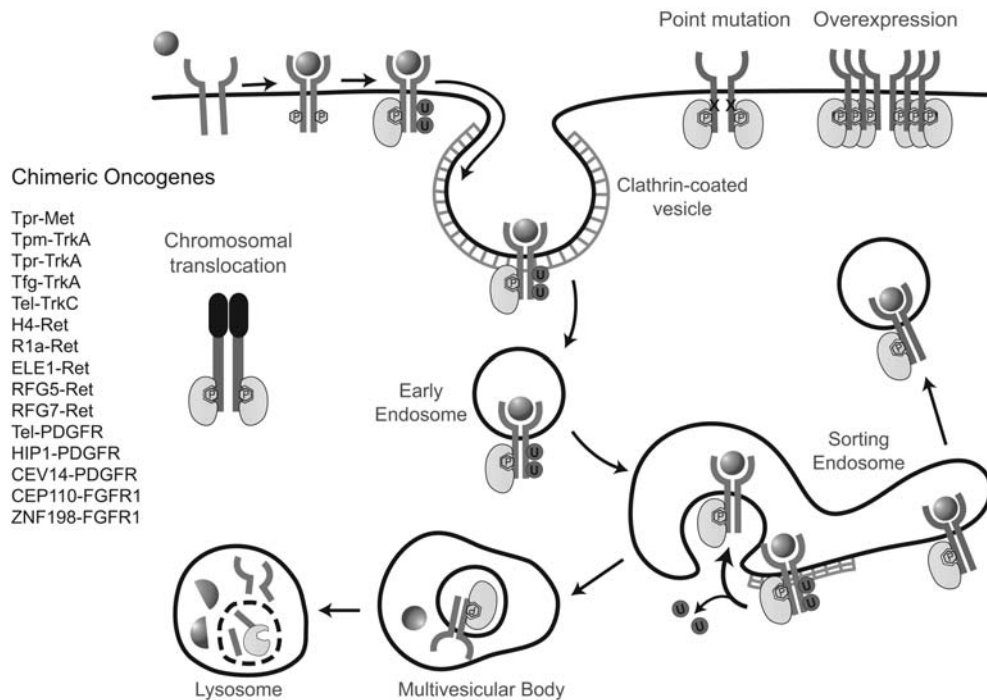


Figure 5. RTK ubiquitination and downregulation. Subsequent to the activation of several RTK's, Cbl is recruited to the receptor and induces receptor ubiquitination. Following internalization, ubiquitinated RTKs are enriched in an endosomal microdomain characterized by a bilayered clathrin coat. Receptors are subsequently internalized in inner vesicles. The process of receptor enrichment and subsequent internalization involves multiple proteins that contain ubiquitin interacting motifs (Hrs, STAM and proteins of the ESCRT complexes) as well as deubiquitinating enzymes (DUBs) that remove ubiquitin moieties from receptors. Fusion of multivesicular bodies with lysosomes leads to the degradation of inner vesicles and their contents by lysosomal proteases. RTKs that are not ubiquitinated can be recycled back to the plasma membrane. RTKs can be dysregulated in human tumors through several mechanisms. This includes amplification and point mutation. Many like Tpr-Met are activated following chromosomal translocation, where in each case a protein dimerization motif is fused to the cytosolic kinase domain of the receptor. These proteins would not be expected to enter the endosomal pathway, and escape lysosomal degradation.

with cell transformation, identified the importance for negative regulation of RTKs to suppress their transforming activity (Peschard et al., 2001a; Peschard and Park, 2003). Multiple mechanisms that reduce Cbl-mediated ubiquitination of RTKs, such as enhanced Cbl degradation, or sequestration, have been identified (Wong et al., 2002; Bao et al., 2003; Wu et al., 2003; Lee et al., 2004). In addition, mutations in RTKs or Cbl proteins that impair Cbl-mediated RTK ubiquitination have been observed in tumours (Peschard and Park, 2003). These observations suggest that loss of a Cbl TKB binding site may be a common mechanism that contributes to full oncogenic activation of RTKs. Moreover RTKs are frequently activated in human tumours following chromosomal translocation (Fig. 5). In general, this fuses a protein dimerization domain with the cytosolic kinase domain of the receptor, resulting in constitutive receptor dimerization and activation (Lamorte and Park, 2001). Over 25 RTK-derived fusion proteins have been identified in human tumours. In most cases, the N-terminal signal peptide, necessary for protein targeting to the membrane, is deleted in the rearranged kinase and where studied, these proteins are cytosolic (Lamorte and Park, 2001). Localization to the cytosol would preclude their entry in the endocytic pathway and hence, their lysosomal targeting and degradation (Fig. 5). However, it remains to be determined whether these oncoproteins are ubiquitinated and targeted for degradation by the proteosomal pathway. Hence, the loss of negative control exerted through Cbl proteins, through chromosomal rearrangements, or mutations that delete Cbl binding sites, may be an important contribution to the deregulation of Met and other RTKs observed in cancers.

Met and cancer therapy

Met was initially identified as an oncogene, and since many studies have now established that Met and/or HGF/SF are inappropriately expressed or activated in many human cancers, it is considered that Met and HGF/SF are important therapeutic targets. In the past few years multiple strategies have been developed to target Met activity and attenuate Met signalling. These include decoy

receptors, and a recombinant Sema domain that competes with HGF binding (Kong-Beltran et al., 2004; Michieli et al., 2004), anti-Met or anti-HGF/SF antibodies, siRNA or ribozymes that target Met or HGF/SF (Date et al., 1997; Cao et al., 2001; Abounader et al., 2002), geldanamycin and derivatives that inhibit molecular chaperone function (Webb et al., 2000), inhibitors that compete for recruitment of key signalling proteins, as well as small molecule inhibitors that target Met catalytic activity (Atabey et al., 2001; Christensen et al., 2003). The widespread expression of HGF/SF and Met in cancers may provide an attractive and possibly general therapeutic target for many human cancers.

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