REVIEW

The HGF receptor family: unconventional signal transducers for invasive cell growth

Paolo M. Comoglio* and Carla Boccaccio

Institute for Cancer Research, University of Torino Medical School, Torino, Italy

The HGF receptor family includes tyrosine kinases encoded by three oncogenes: *MET, SEA* and *RON.* The members of this gene family share a unique functional feature: they mediate cell dissociation and motility ('scattering') in physiological conditions, and invasiveness in their activated versions. The *Met, Ron* and *Sea* receptors display a distinctive signal transduction behaviour. Unlike conventional growth factor receptors, their cytoplasmic tails contain a multifunctional docking site. Upon autophosphorylation, this sequence simultaneously binds and activates multiple SH2-containing transducers, including Ras and PI 3-kinase. A deregulated activation of this 'supersite' triggers a dramatic pleiotropic signal which is responsible for invasive cell growth.

HGF family receptors induce cell 'scattering' and matrix invasion

Met, *Ron* and *Sea* belong to a distinct subfamily of tyrosine kinase receptors, as they share specific structural homologies and biochemical features (Fig. 1). These include: (i) the heterodimeric $\alpha-\beta$ subunit structure (Giordano *et al.* 1989), (ii) two neighbouring tyrosine residues in the kinase domain, responsible for the regulation of enzymatic activity upon autophosphorylation (Ferracini *et al.* 1991), and (iii) a two-tyrosine docking site in the C-terminal tail that mediates high-affinity interactions with multiple SH2-containing signal transducers (Ponzetto *et al.* 1994).

Met, the prototype of the family, is the receptor for HGF (Naldini *et al.* 1991; Bottaro *et al.* 1991). A distinctive property of HGF is its ability to elicit a pleiotropic response which includes growth, cell dissociation, motility and polarization. *Met* transduces growth signals in hepatocytes (Nakamura *et al.* 1986; Gohda *et al.* 1988; Zarnegar & Michalopoulos 1989), kidney tubular epithelia (Harris *et al.* 1993), skin keratinocytes (Kan *et al.* 1991) and melanocytes (Halaban *et al.* 1992). In epithelial cells, the ligand-induced activation of *Met* is followed by a characteristic phenomenon—'scattering'—that involves cytoskeletal reorganization, loss of intercellular junctions and cell-dissociation, followed by active migration (Gherardi *et al.* 1989). Cell 'scattering' is associated with the invasion of extracellular matrices (Stoker 1989; Weidner *et al.* 1990; Giordano *et al.* 1993). Finally, *Met* activation induces cell polarization that leads to the formation of three-dimensional branched tubular structures in epithelial and endothelial cells (Montesano *et al.* 1991; Bussolino *et al.* 1992; Weidner *et al.* 1993). This complex morphogenic response results from the concurrence of cell 'scattering', matrix invasion, proliferation and polarization.

We have recently shown that *Ron* and *Sea* elicit the same array of multiple biological responses including 'scattering', proliferation, cell-polarization and tubule formation (Medico *et al.* 1996). The ligand for *Sea* has not yet been identified, while *Ron* has been identified as the receptor for a factor known as HGF-like or MSP, a rather misleading acronym which stands for macrophage activating protein (Gaudino *et al.* 1994; Wang *et al.* 1994). *Ron* is expressed in a number of cells of epithelial origin, in neurones, and in lineages of haemopoietic origin such as granulocytes, monocytes and osteoblasts (Gaudino *et al.* 1995). Cell scattering and tubulogenesis are not elicited by other tyrosine kinase receptors, such as PDGF or EGF receptors, which are often expressed and functionally active in cells sensitive to HGF or MSP. This indicates that these

* *Corresponding author:* Fax: +39 11 650 9105.

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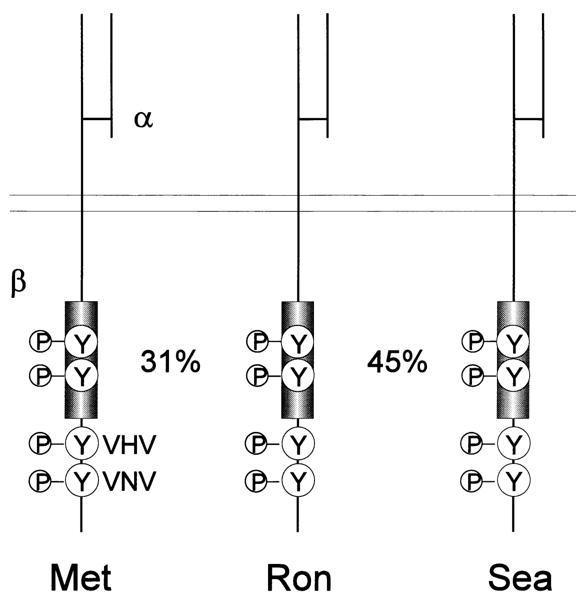

Figure 1 Schematic drawing of the HGF receptor gene family. The family includes three members: *Met*, the prototype, is a heterodimer composed of an extracellular (α) chain, bound to a transmembrane (β) chain by disulphide bonds. *Ron* and *Sea*, share the two-chain structure and the indicated overall homology. Distinctive functional features are conserved in all three receptors: namely the two tyrosines in the kinase domain (rectangle) and the so called two-tyrosine 'supersite' in the C-terminal tail, characterized in the HGF receptor by the sequence YVHVxx-xYVNV.

responses are a distinctive biological feature of the subfamily to which *Met, Ron*, and *Sea* belong (Medico *et al.* 1996; Skeel *et al.* 1991).

Interestingly, HGF and MSP and their receptors are structurally related. Both factors are proteins of high molecular weight (190 kDa), made of an α-chain containing four 'kringle' domains and a disulphide-linked β-chain that is highly homologous to serine-protease, but devoid of catalytic activity (Nakamura *et al.* 1989; Miyazawa *et al.* 1989; Yoshimura *et al.* 1993). HGF is produced by cells of mesenchymal origin widely distributed throughout tissues and organs (Rubin *et al.* 1991), whilst the main source of MSP appears to be the liver. The genes encoding HGF and the HGF-receptor (*MET*), are located in the long arm of chromosome 7 (q11.2-q21.1: Dean *et al.* 1985; Weidner *et al.* 1991). Co-localization also occurs in the genes encoding MSP and *RON*, both mapping to human chromosome 3p2.1 (Han *et al.* 1991; Ronsin *et al.* 1993).

HGF and MSP are secreted as biologically inactive single-chain precursors; maturation into the active $\alpha-\beta$ heterodimer results from proteolytic cleavage mediated by specific convertases. Pro-HGF is activated in tissues by urokinase, an enzyme well known as a promoter of cell migration and matrix invasion (Naldini *et al.* 1992, 1995). In the process of wound healing an alternative pathway is used; blood coagulation activates a 'XII-like factor' protease that cleaves the precursor at the same Arg^{494} site (Miyazawa *et al.* 1994; Shimomura *et al.* 1995). No specific convertases have yet been identified for Pro-MSP.

The kinase activity of *Met* and *Ron* are tightly regulated

Unlike other growth factor receptors, such as those for EGF or PDGF, the catalytic activity of the *Met* kinase is strongly up-regulated by tyrosine auto-phosphorylation (Naldini *et al.* 1991a). The increase in phosphorylation rate is due to a several-fold increase in the V_{max} of the enzyme-catalysed phosphotransfer reaction. Two tyrosines are responsible for this auto-catalytic activation (Y^{1234} and Y^{1235}, Fig. 2), located in the kinase domain and corresponding to the major phosphorylation site (Ferracini *et al.* 1991). Both tyrosines are essential for the full activation of the kinase (Longati *et al.* 1994) and required for unleashing the transforming potential of the oncogene upon overexpression or rearrangement (Zhen *et al.* 1994). The tyrosine doublet is present at homologous locations in the proteins encoded by *RON* and *SEA*, and only in the closest relatives of the family: the insulin and insulin-like receptors (Tornqvist & Avruch 1988).

While autophosphorylation in tyrosine strongly up-regulates the kinase activity of *Met*, negative regulation occurs through two distinct pathways, both resulting in exogenous serine phosphorylation of the receptor cytoplasmic domain. The first is mediated by protein kinase-C (Gandino *et al.* 1990). An independent negative-feedback pathway is triggered by the rise of the intracellular Ca^{2+} concentration; the enzyme involved is a serine kinase with the biochemical properties of Ca^{2+}-Calmodulin kinase III (Gandino *et al.* 1991). Interestingly, the target residue phosphorylated in both instances is Ser^{985} (Fig. 2), located within the juxta-membrane domain of *Met*. This residue is embedded in a canonical consensus sequence for phosphorylation by protein kinase-C and by Ca^{2+}-Calmodulin kinase III (Kennelly & Krebs 1991). The inhibitory effects of either PK-C or calcium ions on HGF receptor activity are lost when the juxta-membrane Ser^{985} is substituted with an alanine residue by site-directed mutagenesis (Gandino *et al.* 1994).

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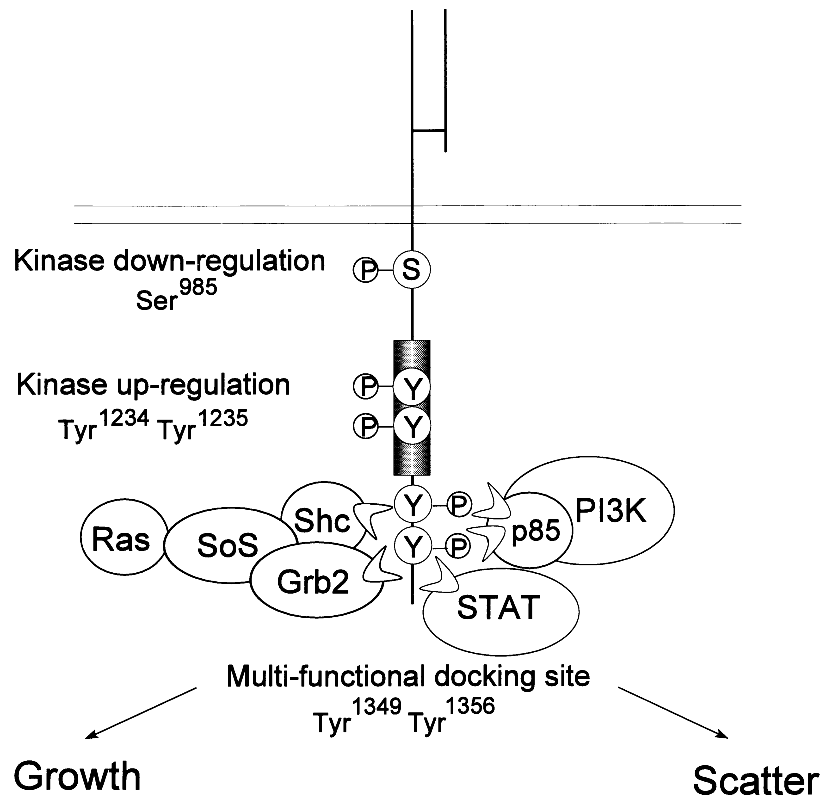

Figure 2 Schematic drawing of the HGF receptor family signaling mechanism. The V_{max} of the receptor kinase activity is increased after auto-phosphorylation of two tyrosines (1234 and 1235) located in the catalytic domain (rectangle). The kinase activity of the receptor is inhibited by phosophorylation of a serine residue (985) in the juxtamembrane domain via PKC or Ca^{2+}-CAM kinase. The multi-functional docking site ('supersite') in the C-terminal tail contains two phosphotyrosines that bind SH2 domain-containing signal transducers. The Ras activator complex SoS-Grb2 specifically binds Tyr^{1356}. Shc can bind both Tyr^{1349} and Tyr^{1356}. The PI3K adaptor subunit p85 contains two SH2 domains, each associating either Tyr^{1349} or Tyr^{1356}. STAT (Signal Transducer and Activator of Transcription) 3 binds to Tyr^{1356}. The transduction pathway mediated by Grb2-Sos-Ras is critical for the stimulation of cell proliferation, while the other signal transducers are mainly involved in activating the scatter programme.

Given the above biochemical properties, the potentially harmful invasive growth response triggered by the *Met* receptor is tightly controlled. Auto-amplification of this response is regulated by negative feedback inhibition, because receptor auto-phosphorylation is followed by activation of phospholipase-Cγ (Ponzetto *et al.* 1994). The latter generates diacyl-glycerol, a powerful activator of protein kinase-C, and inositol-3-phosphate that, in turn, induces a transient increase of $[Ca^{2+}]$ from intracellular stores.

Signal transduction through the multifunctional docking site

As discussed above, HGF is a pleiotropic factor, capable of evoking complex biological responses such as mitogenesis, 'scattering' and morphogenesis: accordingly, its receptor activates multiple signal transduction pathways. The stimulation of responsive cells induces activation of PI 3-kinase (Graziani *et al.* 1991; Ponzetto *et al.* 1993), of a *Ras* nucleotide exchanger (Graziani *et al.* 1993; Hartmann *et al.* 1992) and of a tyrosine phosphatase (Villa-Moruzzi *et al.* 1993). Moreover, upon phosphorylation of the receptor, the cytoplasmic tyrosine kinase $pp60^{Src}$, PLCγ and MAP kinase become phosphorylated on tyrosine and activated (Ponzetto *et al.* 1994). Finally, the phosphorylated receptor binds the *Shc* protein, which works as an 'amplifier' of the motogenic as well as the mitogenic response (Pelicci *et al.* 1995).

Tyrosine kinase receptors, such as those for PDGF or EGF, couple and activate cytoplasmic signaling molecules by auto-phosphorylating tyrosine residues embedded in specific amino acid sequences that become docking sites for conserved structural modules known as 'Src homology 2' (SH2) domains (Cantley *et al.* 1991; Koch *et al.* 1991). SH2 domains are found in one or two copies in molecules involved in signal transduction such as PI 3-kinase, *Ras* GAP, PLCγ, *Src*-related tyrosine kinases, the tyrosine phosphatase SHPTP2 and the *Shc* and *Grb*-2 adaptors. For a review see Pawson (1995). In the above mentioned receptors, distinct phosphotyro-sine residues have been identified which are responsible for binding either PI 3-kinase, PLCγ, *Ras* GAP or $pp60^{c-Src}$. A comparison of the binding sites in different receptors (Cantley *et al.* 1991), as well as a selection of optimal phosphopeptides from a synthetic library by affinity chromatography (Songyang *et al.* 1993), have led to the identification of consensus sequences for individual

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SH2 domains. Moreover, studies based on peptide competitions and site-directed mutagenesis have shown that the amino acids in the +1, +2 and +3 positions—with respect to the phosphorylated tyrosine—are critical in determining the selectivity of the different SH2 domains (Fantl *et al.* 1992; Cantley *et al.* 1991; Rotin *et al.* 1992; Songyang *et al.* 1993; Waksman *et al.* 1992, 1993; Eck *et al.* 1993).

We have shown that, unlike the docking sites identified in other tyrosine kinase receptors, a short sequence located in the *Met* receptor C-terminal tail, and containing two tyrosines that become phosphorylated upon HGF binding, is alone responsible for mediating high-affinity interactions with multiple SH2-containing cytoplasmic effectors (Ponzetto *et al.* 1994). The *Met* sequence $\underline{Y^{1349}VHV}NAT\underline{Y^{1356}VNV}$ (Fig. 2) is able to interact and to activate PI 3-kinase, PLCγ, pp60$^{c\text{-}Src}$, the SHC adaptor and the *Grb-2*/SoS complex. Comparison of the sequence *YVH/NV* with the optimal binding motifs listed by Songyang *et al.* (1993), indicates that it represents a degenerate consensus potentially permissive for multiple SH2 domains. Using synthetic phosphopeptides and a BIAcore biosensor to measure intermolecular binding, we have shown that the SH2 domains of p85, PLCγ and pp60$^{c\text{-}Src}$, interact directly with either version (YVHV or YVNV) of this 'super-site'. *Grb-2*, which has a strong requirement for asparagine in the +2 position (Songyang *et al.* 1993), specifically interacts with the sequence YV$\underline{N}$V. All bindings are characterized by fast association and dissociation rates. When the kinetic parameters measured for the HGF receptor sites were compared to those for the respective optimal sequences, the affinities were similar for all the SH2s studied, with the exception of p85, which showed a K_d one order of magnitude less than the optimal sequence YXXM (Ponzetto *et al.* 1993).

The HGF receptor multi functional 'super-site' represents a variation from the common theme of sequence specificity in the recognition process between SH2 domains and phosphotyrosine residues in tyrosine kinase receptors. To explain how such a site may work, we proposed that a number of factors will concur in determining which transducer binds at any moment. Listed amongst these factors are differences in affinity, variations in local concentration of effectors, and differences in levels of receptor phosphorylation. At very low levels of receptor phosphorylation (early on after binding to the ligand), only the highest affinity interactions will occur, and thus only some transductional pathways will be activated. As the number of phosphorylated receptors increases, the formation of additional complexes involving molecules capable of binding with lower affinities may become possible. Through this mechanism, additional pathways can be superimposed onto those already operating, driving the biological response through sequential steps such as cell dissociation, motility, invasion and growth.

The *Ron* and *Sea* receptors are capable of eliciting the full spectrum of biological responses mediated by the Met receptor, including tubulogenesis, resulting from the concurrence of cell 'scattering', matrix invasion, proliferation and polarization (Medico *et al.* 1996). The overall amino acid identity among the three members of the family is not high (31% between Met and Ron, 37% between Met and Sea). Interestingly, however, the carboxyl-terminal region involved in signal transduction is remarkably conserved. The 'supersite' motif (Y-hydrophobic-X-hydrophobic-X_3-Y-hydrophobic-N-hydrophobic) is present in all three receptors. As also expected, the *Ron* and *Sea* versions of the supersite can interact with the same set of SH2-signal transducers (Ponzetto *et al.* 1994), strongly suggesting that the crucial functional feature of the Met-family of tyrosine kinase receptors is the concomitant activation of multiple signaling pathways.

Concomitant activation of multiple signaling pathways results in invasive cell growth

MET is a potentially harmful oncogene. It is overexpressed in a significant percentage of primary cancers and is amplified in metastases, suggesting a direct involvement in the progression towards malignancy (Giordano *et al.* 1989, 1993; Di Renzo *et al.* 1991; Liu *et al.* 1992; Prat *et al.* 1991). In the thyroid, overexpression is observed in as many as 70% of carcinomas derived from the follicular epithelium (Di Renzo *et al.* 1992; Pierotti *et al.* 1995). In ovarian carcinomas, about 30% of cases show a three to 50-fold increase in expression, with a significant correlation with a particularly aggressive phenotype (Di Renzo *et al.* 1994). In colorectal cancers, the oncogene is overexpressed from five to 50-fold in about 50% of primary lesions, and in 70% of liver metastases (Di Renzo *et al.* 1993). Protein overexpression was found to be associated with amplification of the *MET* gene in only a few primary carcinomas, but in a significant proportion of the metastases examined (Di Renzo *et al.* 1995).

In physiological conditions, the *Met* protein is mainly found in cells of epithelial origin. However, in a fraction

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of osteogenic sarcomas and rhabdomyosarcomas (which are of mesodermal origin) *MET* is aberrantly expressed, and in some instances amplified (Ferracini *et al.* 1995, 1996; Rong *et al.* 1993) Due to the ubiquitous presence of HGF in the surrounding connective tissues, the neoplastic cells acquire a malignant invasive phenotype that can be assessed *in vitro*. In some instances an autocrine loop was detectable, since tumour cells expressing the *Met* receptor were also found to produce HGF, either as a full-size molecule or in the form of the small functional two-kringle variant (Ferracini *et al.* 1996; Rong *et al.* 1994).

The high incidence of MET overexpression in primary cancers and of gene amplification at later stages, in metastases, suggests that the signals transduced by the activated tyrosine kinase via the multifunctional docking site contributes to favourable growth properties for neoplastic cells at the primary site and a selective advantage for the acquisition of metastatic potential. This conclusion is supported by the observation that cells transfected with an activated version of the *MET* gene acquire invasive and metastatic properties, both *in vitro* and in experiments in nude mice (Giordano *et al.* 1993). The key role played by the 'supersite' is shown by the elimination of the two tyrosines in the tail sequence Y^{1349}VHVNATY^{1356}VNV. Although the *Met* kinase is activated constitutively, the cells revert to the normal phenotype (Ponzetto *et al.* 1994). A single amino acid substitution in the *Met* docking site, critical for recruiting the *Grb2/SoS* complex to Y^{1356}, generates a mutant able to activate the PI 3-kinase but not the *Ras* pathway. This mutation does not affect cell scattering and matrix invasion, rather it abolishes transformation. In a mirror experiment, an engineered docking site which binds two *Grb2*/SoS molecules with higher affinity, generates a *Met* mutant that is extremely efficient in activating the *Ras* pathway, but is unable to recruit PI 3-kinase. This mutant is highly transforming but totally unable to support the metastatic phenotype (Giordano *et al.* 1996, submitted). It is concluded that the invasive-metastatic growth induced by *Met*, and by the other members of the family, relies on the integrity of the docking 'supersite' and requires the concomitant activation of multiple signaling pathways.

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