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Review

Structural insights into Met receptor activation

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ABSTRACT

The receptor tyrosine kinase Met plays a pivotal role in vertebrate development and tissue regeneration, its deregulation contributes to cancer. Met is also targeted during the infection by the facultative intracellular bacterium *Listeria monocytogenes*. The mechanistic basis for Met activation by its natural ligand hepatocyte growth factor/scatter factor (HGF/SF) is only beginning to be understood at a structural level. Crystal structures of Met in complex with *L. monocytogenes* InlB suggest that Met dimerization by this bacterial invasion protein is mediated by a dimer contact of the ligand. Here, I review the structural basis of Met activation by InlB and highlight parallels and differences to the physiological Met ligand HGF/SF and its splice variant NK1.

Met and its ligands

Met

A transforming variant of the *met* proto-oncogene was first cloned from chemically mutagenized cells (Cooper et al., 1984) and later shown to encode a tyrosine kinase (Dean et al., 1985) with similarity to growth factor receptors (Park et al., 1987). Subsequently hepatocyte growth factor/scatter factor (HGF/SF) was identified as the Met ligand (Bottaro et al., 1991). Stimulation of the Met receptor induces various cellular responses including proliferation, survival, motility and morphogenesis, i.e. formation of branched tubular structures (Birchmeier et al., 2003). Met is evolutionarily rather recent and its signaling is essential during vertebrate development (Birchmeier and Gherardi, 1998). In the adult, Met signaling is essential for liver and skin regeneration (Borowiak et al., 2004; Chmielowiec et al., 2007; Huh et al., 2004) and has also been implicated in stem cell mediated regeneration of the heart (Linke et al., 2005; Urbanek et al., 2005). Overactivation of Met is implicated in invasive growth of tumor cells and the promotion of cancer metastasis (Benvenuti and Comoglio, 2007; Boccaccio and Comoglio, 2006), making Met an interesting target for the development of therapeutic inhibitors (Eder et al., 2009; Mazzone and Comoglio, 2006; Peruzzi and Bottaro, 2006).

Met is produced as a 1390 residue single-chain precursor that is cleaved by furin into an N-terminal, completely extracellular α-chain and the membrane-spanning β-chain that, in addition to the cytoplasmic tyrosine kinase domain, also forms large parts of the ectodomain (Fig. 1) (Giordano et al., 1989; Tempest et al., 1988). Upon ligand stimulation Tyr1234 and Tyr1235 in the activation loop of the tyrosine kinase domain (Longati et al., 1994) and Tyr 1349 and Tyr1356 in the substrate docking site are phosphorylated (Ponzetto et al., 1994). The adaptor protein Gab1 plays a central role in Met signal transduction (Sachs et al., 2000). Downstream signaling includes among others the Ras/ERK and the PI3K/Akt pathways. Intracellular signaling will not be discussed here in detail, but has been reviewed elsewhere (Birchmeier et al., 2003; Bolanos-Garcia, 2005; Furge et al., 2000).

HGF/SF

The double name of HGF/SF for the Met ligand derives from its independent identification as mitogen for hepatocytes (Miyazawa et al., 1989; Nakamura et al., 1989) and as a motility factor for epithelial cells (Stoker et al., 1987). HGF/SF is structurally related to plasminogen, the precursor of the blood-clotting protease plasmin (Miyazawa et al., 1989; Nakamura et al., 1989). HGF/SF consists of an N-terminal hairpin (N), four kringle (K1-K4) and a C-terminal serine-protease homology (SPH) domain (Fig. 1). HGF/SF is enzymatically inactive due to mutations in active-site residues. Like plasminogen that is converted to the active form plasmin by proteolytic cleavage, HGF/SF needs to be converted from a non-activating

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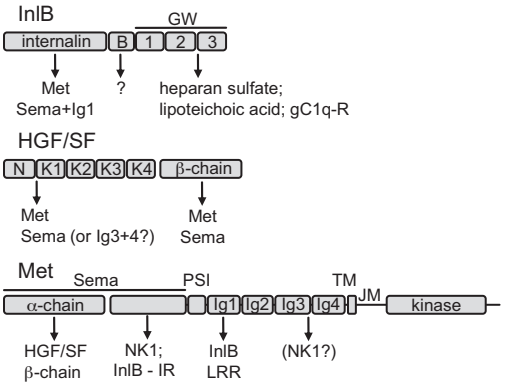

Fig. 1. Domain structure of InlB, HGF/SF and Met. Binding partners of individual domains are indicated.

(but still Met-binding) single-chain form into an active two-chain form. The N and K1–K4 domains form the α-chain, the serine protease homology domain forms the β-chain. Both chains stay connected by a disulfide bridge. Two shorter splice variants exist, namely NK1 that acts as a heparin-dependent partial Met agonist and NK2, a Met antagonist (Chan et al., 1991; Cioce et al., 1996). The structure–function relationship of individual HGF/SF domains and their combinations is complex (Holmes et al., 2007) and only some aspects will be discussed in this review.

InlB

For a long time HGF/SF was the only known Met ligand. This changed when Met was identified as a receptor for the *Listeria monocytogenes* invasion protein InlB (Shen et al., 2000). *Listeria* is a ubiquitous Gram-positive bacterium and pathogen for humans. Being a facultative intracellular bacterium, it expresses two invasion proteins called internalin (InlA) and InlB that bind to specific host cell receptors (E-cadherin and Met, respectively) and induce uptake of bacteria into normally non-phagocytic cells (Hamon et al., 2006). InlB-dependent uptake requires the endocytic machinery and depends, among others, on clathrin and dynamin (Veiga and Cossart, 2005; Veiga et al., 2007). This involves ubiquitination of Met by the ubiquitin ligase Cbl, which is also involved in Met down-regulation by endocytosis upon stimulation by HGF/SF (Petrelli et al., 2002).

InlB belongs to the extended family of internalin proteins found in *Listeria* (Bierne et al., 2007). Internalins are characterized by an internalin domain that consists of a central leucine-rich repeat (LRR) region flanked by specialized capping structures (Schubert and Heinz, 2003). A helical structure forms the N-terminal cap region (Marino et al., 1999), while the C-terminal inter-repeat (IR) region folds into an immunoglobulin-like β-sandwich (Schubert et al., 2001). In addition to the internalin domain, InlB harbors a central B-repeat and three C-terminal GW-domains, named after a GlyTrp dipeptide (Fig. 1). The cap-LRR fragment is sufficient and required for Met binding (Shen et al., 2000), but cannot induce Met phosphorylation in its monomeric form (Banerjee et al., 2004). Addition of the IR region to the cap-LRR fragment results in a protein that can induce Met phosphorylation and downstream signaling, but not a cellular response (Banerjee et al., 2004; Niemann et al., 2007).

The B-repeat is poorly characterized. It has been reported to lead to hyperactivation of the Ras/ERK pathway without enhancing Met activation, when combined with the internalin domain (Copp et al., 2003). This suggested that it might bind an as yet unidentified co-receptor. The GW domains are structurally related to SH3 domains (Marino et al., 2002). They are highly basic and interact with polyanions. The GW domains are responsible for the non-covalent attachment to the bacterial cell surface (Braun et al., 1997) through binding to lipoteichoic acid (Jonquieres et al., 1999). Heparan-sulfate and potentially other glycosaminoglycans compete with this interaction (Marino et al., 2002). The GW domains alone do not stimulate uptake, but they synergize with the Met-binding internalin domain (Banerjee et al., 2004). A construct lacking the GW domains is less effective in stimulating Met phosphorylation than full-length InlB (Banerjee et al., 2004; Ferraris et al., 2010). Likewise, cells deficient in general glycosaminoglycan synthesis or specifically in synthesis of heparan-sulfate are invaded by *Listeria* less efficiently than wild-type cells (Jonquieres et al., 2001)

Decorin

Lately the small proteoglycan decorin was reported to bind to Met with nanomolar affinity resulting in transient phosphorylation of tyrosines in the activation loop of the Met kinase domain, Tyr1003 in the juxtamembrane region and Tyr1356 in the multi-docking site (Goldoni et al., 2009). In contrast, Tyr1349, the second tyrosine in the multi-docking site, is not phosphorylated. As a result, decorin binding leads to Met ubiquitination and down-regulation by endocytosis rather than activation of downstream signaling pathways.

Common themes and structural peculiarities in RTK activation

General concepts

In order to put the data on ligand binding and receptor activation for Met into a broader perspective, I will briefly outline general concepts of ligand mediated receptor tyrosine kinase (RTK) activation and then describe four examples for which crystal structures have provided a good structural understanding of ligand-mediated receptor dimerization. With only one transmembrane helix, monomeric RTKs have no possibility to transduce a signal over the membrane by a conformational change. Early on, it was proposed that ligand binding causes dimerization or more generally oligomerization of the receptor to generate a signal responsible for cross-phosphorylation of the intracellular kinase domains, sometimes also referred to as autophosphorylation (Schlessinger, 1988; Ullrich and Schlessinger, 1990). Ligand-induced dimerization proved to be a useful and general principle of RTK activation, but the structural mechanisms underlying dimer assembly are far more diverse than had been anticipated. Dimerization is called ligand-mediated when the dimer contact is formed by the ligand. If the dimer contact is formed by the receptor itself, we talk about receptor-mediated dimerization. In addition, there are 2:2 assemblies that fit neither of these categories and sometimes GAGs like heparin or heparan-sulfate contribute to stabilization of receptor dimers.

Ligand mediated dimerization by constitutively dimeric ligands

In the most straightforward case, the ligand is a constitutive, 2-fold symmetric homodimer with two identical receptor binding sites (Fig. 2A). The binding of vascular endothelial growth factor (VEGF) to its receptor Flt1 represents one example of this ligand-mediated dimerization (Wiesmann et al., 1997) and the binding of stem cell factor (SCF) to its receptor Kit is a second one (Liu et al., 2007). The binding site for one receptor may be formed by one protomer of the ligand dimer as in the case of SCF, or by both protomers as in the case of VEGF. However, the picture of a solely ligand-mediated dimerization is oversimplified in the cases men-