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Signalling networks in focus

Met endosomal signalling: In the right place, at the right time

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ABSTRACT

Deregulated signalling of the Receptor Tyrosine Kinase (RTK), Met, and/or its ligand HGF have been associated with cancer formation and progression to metastasis, with Met/HGF often overexpressed or mutated. Thus, Met has become a major target for cancer therapy and its inhibition is currently being tested in the clinic. It has recently become evident that, instead of signalling at the plasma membrane only, Met signals post-internalisation from endosomal compartments. Thus, Met endocytic trafficking is required for the full activation of signals such as Gab1, ERK 1/2, STAT3 and Rac1, all implicated in cell survival, invasion and metastasis. Modifications in the balance between degradation and recycling of Met may also impinge on Met signalling. Moreover, oncogenic Met mutations in the kinase domain trigger constitutive Met internalisation/recycling, leading to "endosomal signalling" and consequent cell transformation. Using Met as an example, this review outlines the evidence that the molecular mechanisms regulating trafficking and endosomal signalling may be exploited to design future cancer therapies.

Signalling networks facts

- Met and its ligand HGF, through promoting multiple cell functions such as cell proliferation, survival and motility, play a role in maintaining normal tissue homeostasis but also in disease progression such as cancer, especially when Met is overexpressed or mutated.
- HGF binding to Met triggers: (1) the activation of several signalling pathways including ERK1/2, AKT and STAT3; (2) dynamin and clathrin dependent Met endocytosis followed by further intracellular trafficking, progressive degradation and possible recycling back to the plasma membrane for a certain proportion of Met.
- Met signalling and Met endocytic trafficking happen concomitantly. Thus Met continues to signal during endocytic trafficking and this plays a role in Met-dependent cell migration, anchorage independent growth and tumourigenesis.

1. Introduction

The Receptor Tyrosine Kinase, Met, was first discovered as the TPR-MET oncogenic fusion protein in 1984 by Cooper et al. (Cooper et al., 1984). A few years later Met was found to belong to the family of RTKs (Park et al., 1987). It is a proto-oncogene and is found expressed on the surface of epithelial and endothelial cells, where it binds specifically to its only known ligand, hepatocyte growth factor (HGF). Met and its ligand HGF are involved in many normal and pathological biological processes.

Met and HGF expression are necessary during foetal development as mice lacking either die *in utero* (Bladt et al., 1995; Schmidt et al., 1995; Uehara et al., 1995). In adult life, expression of HGF and Met often increases in injured tissues (Michalopoulos and DeFrances, 1997; Nakamura et al., 2000) and Met expression in keratinocytes has been shown to be required for wound healing (Chmielowiec et al., 2007). Furthermore, Met signalling is also involved in angiogenesis *in vitro* and *in vivo* (Bussolino et al., 1992; Grant et al., 1993). As Met can activate many downstream signalling pathways, initiating many cellular functions, deregulation of Met signalling can lead to the formation and progression of various cancers and may therefore be an important target for therapy (Gherardi et al., 2012; Trusolino et al., 2010).

2. Functions of the Met signalling pathway

The initiation of Met signalling begins with the binding of HGF to the Met receptor at the plasma membrane. This leads to the stable dimerisation of two molecules of Met and enables transautophosphorylation of the tyrosine kinase domain at tyrosine residues Y1234 and Y1235, followed by transphosphorylation of the two tyrosine residues Y1349 and Y1356 in the C-terminal region (Fig. 1a and b). These two tyrosines form the "multisubstrate docking site", which is unique to members of the Met subfamily and essential for Met signalling. It enables Met to bind to multiple

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substrates and activate a variety of signalling pathways either through direct interaction with signalling molecules or through adaptors such as growth factor receptor-bound protein 2 (Grb2) and Grb2-associated-binding protein 1 (Gab1) (Fig. 1c). In this review we will focus only on the few Met pathways, which have been linked to Met trafficking. For a more comprehensive review of Met signalling see (Trusolino et al., 2010)

Gab1 binds to activated Met on either Y1349 or Y1356 through its specific 'Met binding site' or indirectly through the adaptor Grb2. Gab1 contains multiple tyrosine sites that, when phosphorylated, mediate the additional binding of proteins including Phosphatidyl Inositol 3-kinase (PI3K), CT10 Regulator of Kinase (Crk), phospholipase Cγ (PLCγ), SRC homology 2 domain-containing phosphatase 2 (Shp2) and p120-Ras-GAP (Trusolino et al., 2010). Activation of Gab1 has been shown to encourage tubulogenesis and the migration of muscle precursor cells during embryonic development (Sachs et al., 2000; Lock et al., 2002). In fact, Gab1−/− mice exhibit similar phenotypes to Met-/- mice *in vivo*, being embryonically lethal between E13.5 and E18.5 and displaying placental defects as well as a reduced liver size, demonstrating that Gab1 is essential for Met signalling (Sachs et al., 2000).

Extracellular signal related kinase 1 and 2 (ERK1/2), members of the mitogen-activated protein kinase (MAPK) cascade, are activated by Met *via* several pathways; Gab1-Shp2-Ras (Maroun et al., 2000; Montagner et al., 2005), Grb2-SOS-Ras (Ponzetto et al., 1994) or SHC-Grb2-SOS-Ras (Pelicci et al., 1995). The activation of ERK1/2 is involved in cell survival (Xiao et al., 2001), tubulogenesis (O'Brien et al., 2004) and motility (Kermorgant et al., 2004) downstream of Met and has been specifically shown to be involved in Met dependent liver regeneration (Trusolino et al., 2010; Borowiak et al., 2004).

Rac, a member of the small Rho GTPase family, can be activated by Met through Grb2-SOS-Ras or through the activation of PI3K and is an important regulator of Met dependent actin reorganisation, migration and tubulogenesis (Ishibe et al., 2004; Ridley et al., 1995).

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that can be activated through direct binding to Met. It plays an important role in Met-dependent anchorage-independent growth (Zhang et al., 2002), wound healing (Kermorgant and Parker, 2008), tubulogenesis (Boccaccio et al., 1998) and tumourigenesis (Zhang et al., 2002).

3. Met signalling cascades – the link between Met trafficking and Met signalling

Upon ligand binding, Met undergoes rapid endocytosis and traffics through peripheral endosomes (at around 15 min in HeLa cells) to accumulate on perinuclear endosomes (at around 60–120 min in HeLa cells). Traditionally, it has been thought that receptors only signal from the plasma membrane and get "switched off" through internalisation and subsequent degradation. However, as has been shown for other RTKs such as EGFR (Miaczynska et al., 2004), PDGFR (Miaczynska et al., 2004) and the insulin receptor (Ceresa et al., 1998), following endocytosis, Met can signal from endosomes. Moreover, its endocytosis and sorting to specific endosomes play an important role in its signalling (Kermorgant et al., 2004; Kermorgant and Parker, 2008). In this part (illustrated in

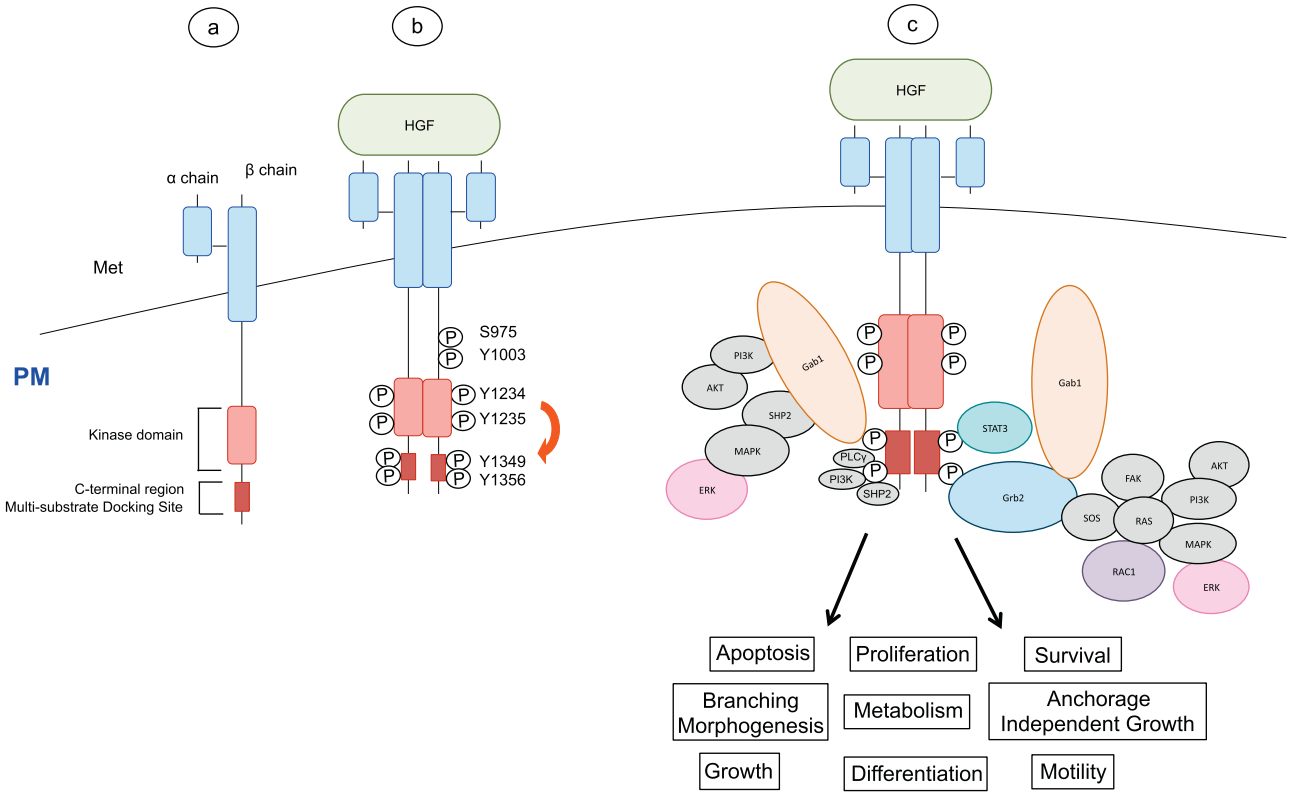

Fig. 1. Met activation and downstream signalling. (a) Met exists as a transmembrane receptor consisting of an α and β chain that are linked by disulphide bonds. (b) Binding of HGF leads to dimerisation of two Met molecules, autophosphorylation of two tyrosine residues (Y1234-5) in the kinase domain and transautophosphorylation of two residues (Y1349-56) in the C-terminal Met docking site. The juxtamembrane domain contains two residues that negatively regulate Met: the tyrosine 1003 (which, when phosphorylated, binds to c-Cbl leading to c-Met degradation) and the serine 985 (which, when phosphorylated, downregulates c-Met kinase activity). (c) Phosphorylation of Met leads to the activation of multiple signalling molecules either through direct binding or through the adaptors Gab1 and Grb2. This leads to the activation of many signalling pathways that, in turn, induce the various cellular processes outlined.