Hepatocyte Growth Factor
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Nutritional Sciences

Molecular Structure and Receptor Type

Hepatocyte Growth Factor (HGF) is a glycoprotein which is expressed mainly by fibroblasts and by other non-parenchymal cells such as macrophages. The cytokine molecule is a heterodimer consisting of a 69kD α-subunit, containing 4 kringle domains and an N-terminal hairpin domain, and a 34kD ß-subunit, containing a domain similar to serine proteases (Figure 1) (Mizuno & Nakamura, 2007). HGF is cleaved from a single chain 728 amino acid precursor called pro-HGF, which is found mainly in the extracellular matrix and on the surface of cells (Stuart *et al*, 2000). Serine proteases convert pro-HGF to the active two chain form (Funakoshi & Nakamura, 2003).

The receptor for HGF is an oncogene product called c-MET. c-MET is a membrane bound heterodimer consisting of a 50kD α-subunit and a transmembrane 145kD ß-subunit. The ß-subunit includes a tyrosine kinase domain on the intracellular side which is activated by the binding
of HGF (Mizuno & Nakamura, 2007).

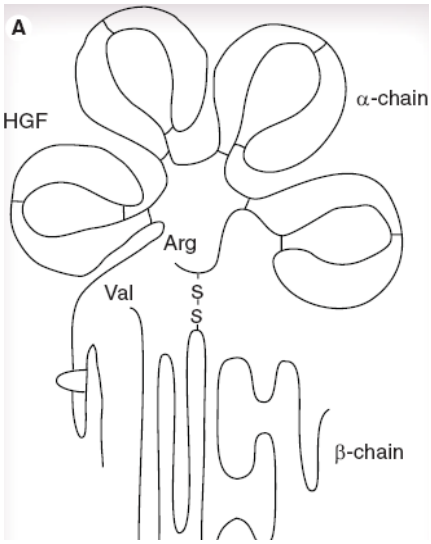

Figure 1: Hepatocyte Growth Factor (Mizuno & Nakamura, 2007)

Mechanisms of Action

HGF has many different mechanisms of action on many different cell types. These actions include cell proliferation, motogenesis (movement and scattering of cells), morphogenesis (formation of branching tubules) and inhibition of apoptosis (Funakoshi & Nakamura, 2003). These fundamental roles in cell cycling mean that HGF plays an essential role in mammalian organogenesis (Boros & Miller, 1995) and organ maintenance, angiogenesis (Mizuno & Nakamura, 2007) and is implicated in the invasion and metastasis of tumour cells in many types of cancer (Grierson *et al*, 2000). The different mechanisms of action of HGF are mediated by different intracellular signalling cascades rather than different receptors on target cells. A broad overview of the actions of HGF, along with some of the different intracellular signalling mechanisms, is shown in Figure 2.

Intracellular responses to the binding of HGF

The tyrosine kinase domain of c-MET binds phosphotyrosine –containing proteins when the receptor has been phosphorylated and activated by HGF. The domain consists of two tyrosine residues, Tyr1349 and Tyr1356, and is termed the multifunctional docking site (Ponzetto *et al*, 1994). This site allows the c-MET receptor to recruit many different cytoplasmic transducers to the cell membrane via their Scr homology 2 (SH2) domains (Cantley *et al*, 1991). The intracellular signalling molecules recruited include phospholipase c-γ (PLC-γ), phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K), Ras G protein, and STAT (a transcription factor) as illustrated in Fig. 2. Others include Shp2 (a tyrosine phosphatase) and Crk (an adapter protein) (Funakoshi & Nakamura, 2003). In addition, amplifying molecules Grb2 and Shc bind to the phosphorylated receptor and increase the cellular response to HGF. They provide more tyrosine-phosphorylated sites for the cytoplasmic transducers already mentioned to bind, and Grb2 provides a binding site for the docking protein Gab1 to associate with the c-MET receptor (Mood *et al*, 2006). Gab1 is the main substrate for c-MET and is key to the activation of downstream signalling pathways (Bertotti & Comoglio, 2003). Gab1 provides a binding site for PLC-γ, which cannot bind directly to the receptor tail (Comoglio, 2001). It also provides sites for PI3K, Shp2, STAT and Crk to associate (Gual *et al*, 2000). Hence the binding of HGF to c-MET induces many different intracellular signalling pathways which lead to different phenotypic outcomes. The downstream pathways that lead to different cellular responses are discussed in detail below.

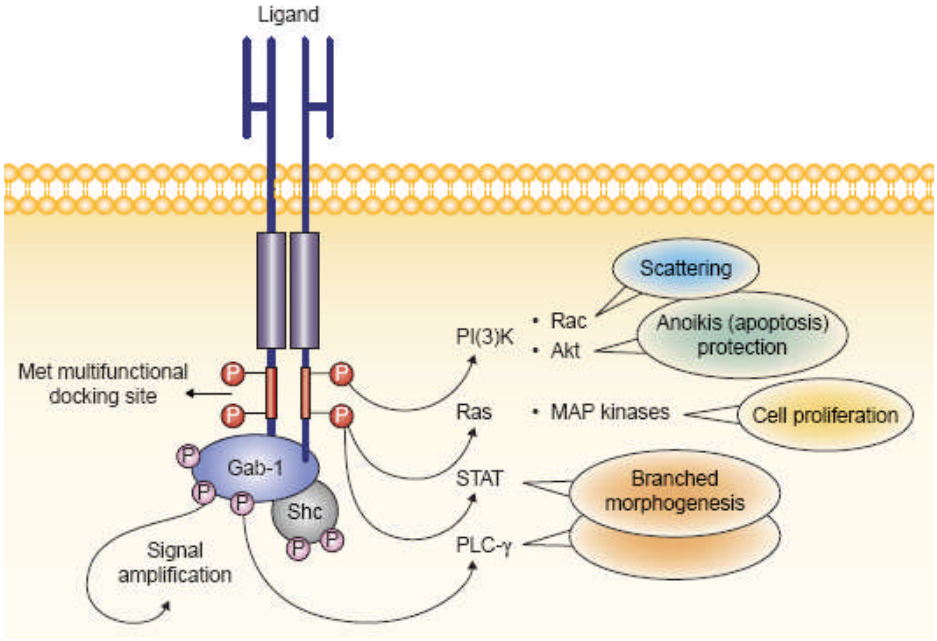

Figure 2: The tyrosine kinase domain of c-MET is phosphorylated by the binding of HGF (shown as 'ligand'). Downstream signalling molecules are recruited by the phosphorylated tyrosine kinases to produce a variety of cellular responses (Comoglio, 2001).

Cell proliferation

HGF is responsible for cell proliferation in both the developing foetus and in the adult during tissue regeneration. Despite its name, HGF stimulates growth and proliferation of many different epithelial cells within different organs of the body. The intracellular signalling cascade responsible for this role is the Sch-Grb2-SOS-Ras pathway (Trusolino & Comoglio, 2002). As already mentioned, Sch and Grb2 are adapter molecules recruited to the phosphorylated c-MET receptor. This brings the adapter molecules to within close proximity of the membrane bound Ras protein. The Ras protein binds guanine nucleotides and is associated to GDP when it is in its inactive state (Graziana *et al*, 1993). Grb2 forms a complex with the nucleotide exchange factor SOS; the Grb2-SOS complex is then able to assist the exchange of GDP for GTP thus activating the Ras protein. The activated Ras protein then activates a cytosolic protein called Raf, which subsequently phosphorylates and activates MEK, a cytosolic kinase. MEK then activates mitogen-activated protein kinase (MAPK) by phosphorylation; these kinases are then able to move into the nucleus where they alter gene expression (Diehl & Rai, 1996). MAPKs phosphorylate and therefore upregulate the activity of transcription factors which control cell growth. The transcription factors targeted include c-myc, Elk-1 and C/EBPß (Hill & Treisman, 1995).

Another intracellular pathway that has been implicated in cell proliferation is the PLC-γ signalling cascade. PLC-γ is phosphorylated and activated by binding to Gab1. The active PLC-γ causes the formation of inositol-1,4,5 triphosphate (IP3) and diacylglycerol (DAG) by hydrolysis of phosphatidylinositol-4,5-bisphosphate. Calcium is released from internal stores in response to stimulation by IP3 (Gual *et al*, 2000). The nuclear membrane in hepatocytes is likely to be permeable to calcium, and an increase in intranuclear calcium may activate calmodulin, calcium-dependent endonucleases or nuclear protein kinase C (PKC). These molecules are involved in regulating cell proliferation (Waybill *et al*, 1991). DAG is also an activator of PKC (Gual *et al*, 2000).

Cell motility

HGF is involved in mediating the complex process of cell motility. In order to migrate, the actin cytoskeleton must extend at the lamellipodia and form adhesions with the extracellular matrix, meanwhile the trailing edge of the cell must detach (Potempa & Ridley, 1998). Ras activation is important in the remodelling of the actin cytoskeleton. As discussed, Ras activation results in the activation of MAPKs. The Raf protein activated by Ras in turn activates p42/p44 MAPK, inducing the loss of adherin junctions which attach cell membranes to the cytoskeleton. This is an early step in the motogenic process (Potempa & Ridley, 1998).

MAPK also induces expression of matrix metalloprotease-9 and uPA which are responsible for degrading components of the extracellular matrix, therefore facilitating cell migration (Comoglio & Boccaccio, 2001).

The heterodimeric protein PI3K also plays an important role in HGF induced cell motility. PI3K is composed of a regulatory subunit, p85, and a 110kD catalytic domain. p85 contains two SH2 domains which mediate binding of PI3K to c-MET or Ras activated protein. Binding activates the 110kD catalytic subunit which has lipid kinase activity. PI3K is in close proximity of the lipid membrane when it is bound to c-MET; it has been suggested that the lipid kinase activity of PI3K may induce vesiculation and aid the trafficking of vesicles to the nucleus which may induce motogenesis (Cantley & Cantley, 1995). PI3K is also implicated in the remodelling of the actin cytoskeleton. PI3K activates a GTP-ase protein, Rac, via interaction with Tiam1, a guanine nucleotide exchanger. Tiam1 then activates p21-activated kinase (PAK). Rac and PAK are involved in the formation of lamellipodia and membrane ruffles which are crucial to cell motility as I described above (Sander *et al*, 1998).

Morphogenesis

An important function of HGF is its role in the formation of branching tubules. Branching tubules are found in many organs of the body and essential for the exchange of materials, for example, the kidney tubules are responsible for processing filtrate and producing urine. Tubule formation occurs during organogenesis and organ maintenance and is mediated by HGF (Bertotti & Comoglio, 2003). Tubule formation is triphasic, cell scattering and cell growth must occur via the mechanisms I have already described before the third phase of tubulogenesis can occur (Boccaccio *et al*, 1998). Tubule formation depends on the binding and sustained activation of Gab1 to the multifunctional docking site of c-MET. Gab1 recruits PLC-γ and Shp2 which are essential in tubule formation. It is suggested that PLC-γ is necessary for anchorage independent growth (Gual *et al*, 2000). The transcription factor STAT3 is also vital for tubulogenesis. STAT3 can bind directly to the c-MET receptor or indirectly via Gab1 by its SH2 domain. This induces tyrosine phosphorylation of STAT causing the molecule to dimerise and translocate to the nucleus. Once inside the nucleus STAT3 binds to the promoter regions of the *c-fos* and *waf-1* genes inducing their expression. These genes are responsible for stopping cell growth and inducing cell differentiation, therefore stimulating morphogenesis (Boccaccio *et al*, 1998). The unique ability of HGF to induce branching tubulogenesis is likely to involve integration of these intracellular signalling pathways (Boccaccio *et al*, 1998).

Survival

The PI3K and PLC-γ pathways are implicated in producing anti-apoptotic mechanisms within the cell. As previously described, activation of PLC-γ results in the release of calcium from intracellular stores. Cell survival depends on the calcium equilibrium between the mitochondria and the endoplasmic reticulum therefore calcium release via PLC-γ activation may be anti-apoptotic (Gual *et al*, 2000). In addition, the activation of the 110kD lipid kinase domain of PI3K as previously described results in the formation of lipid products. The lipids produced act as messengers and activate Akt which in turn phosphorylates BAD (Porter & Vaillancourt, 1998). This acts as an anti-apoptotic signal and may mediate survival by maintaining cell anchorage (Gual *et al*, 2000).

HGF and cancer

HGF plays an important role in the progression of many types of cancer due to its fundamental roles in cell proliferation, motility, morphogenesis and survival. The multifunctional capacity of HGF and c-MET means that all the requirements for invasive growth and metastasis are integrated within one signal, therefore aberrant activation of this signal due to mutation can result in malignancy. Signal transducers within HGF activated intracellular pathways are therefore a target for developing diagnostic and therapeutic strategies in the treatment of cancer (Trusolino & Comoglio, 2002).

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