# Gene of the month: HGF

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Gene of the month: \textit{HGF}

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INTRODUCTION

Hepatocyte growth factor (HGF) is a multifunctional cytokine with important roles in cell proliferation, survival, motility and morphogenesis. Secreted by cells of mesenchymal origin, HGF is the specific ligand for the tyrosine-kinase receptor c-MET (cellular mesenchymal-epithelial transition), also called MET, which is expressed in different types of epithelial, endothelial and haematopoietic progenitor cells. The HGF/MET axis is involved in several biological processes, such as embryogenesis, organogenesis, adult tissue regeneration (including wound healing and liver regeneration) and carcinogenesis, for both solid and haematological malignancies.[1-2] HGF and its particular interaction with the MET receptor have been extensively investigated in the last decades and remain the focus of numerous clinical trials.[3-8] This short review focuses on HGF structure and function, as well as its roles in liver regeneration and different types of tumour.

HGF STRUCTURE

HGF is mapped on the long arm of chromosome 7 at q21.1 and it is formed by 18 exons, interrupted by 17 introns, spanning 71,433 bases of genomic DNA. It encodes the inactive pre-pro-HGF, a single chain of 728 amino acids (83134 Da), which includes a signal sequence (1-31), a heavy alpha-chain (32-494; 69kDa) and a light beta-chain (495-728; 34kDa). The first exon contains the signal peptide and a 5'-untranslated region. The following ten, twelve and six remaining exons encode the alpha-chain, with four kringle structures, the short spacer region between the alpha- and beta-chains and the beta-chain, respectively.[9-11] HGF primary structure was determined in 1989, but multiple transcript variants encoding different isoforms have been identified by alternative splicing of the gene since then.[12]
The inactive pre-pro-HGF becomes active after a two-cleavage process. Firstly, the signal peptide of the pre-pro-HGF is degraded, generating the pro-HGF, which is also cleaved between Arg494 and Val495. Several serum or cell-membrane proteases have been described to be involved, such as HGF activator (HGF-A), urokinase-type plasminogen activator, plasma kallikrein, coagulation factors XII and XI, matriptase and hepsin.[13]

Among them, HGF-A is the main protease responsible for the activation of pro-HGF in serum. The activation process of HGF plays an important role in the regulation of tissue regeneration and susceptibility to pathological conditions. Although HGF-A knock-out mice showed normal development, they have impaired restitution of epithelia after mucosal injury.[14] Similarly, fibroblasts from patients with idiopathic pulmonary fibrosis have been shown to have lower capacity to activate HGF compared with control fibroblasts.[15]

The final active heterodimeric molecule is produced by a disulphide bond between the alpha and beta chains. Although this multifunctional protein belongs to the plasminogen subfamily of S1 peptidases, no proteolytic activity has been detected, probably due to the substitution of two out of three amino acids required in the catalytic triad.[16] It has been suggested that HGF could be evolutionally derived from those encoding proteases implicated in the coagulation cascade and fibrinolysis. Indeed, not only is its organisation very similar, but its products are also. The HGF alpha-chain has 38% homology with plasmin, and the beta-chain is structurally similar to the catalytic domains of serine proteases, but with amino acid substitutions in the active site.[1]
In contrast to other growth factors and their receptors, HGF binds exclusively to the product of the c-MET proto-oncogene, also mapped on chromosome 7.[17-19] The MET receptor is a 190kDa protein, comprising a ligand-binding extracellular domain, a transmembrane region and a cytoplasmic domain with tyrosine kinase activity. Although HGF alfa-chain has a higher affinity for MET, it is the beta-chain which activates the receptor.[20 21] Upon MET dimerization, kinase activation results in auto-phosphorylation of tyrosines Y1349 and Y1356, and recruitment of several substrates, including growth factor receptor-bound protein 2, Shc, p85 subunit of phosphatidylinositol 3’ kinase, phospholipase Cγ, signal transducer and activator of transcription 3 (STAT3) and Grb2-associated binding protein 1. The downstream signalling pathways generate diverse cellular responses, such as proliferation, survival, motility, invasion and stimulation of angiogenesis (Figure 1).[22-34]

Several growth factors, cytokines and prostaglandins upregulate HGF gene expression: b-FGF (basic fibroblast growth factor), OSM (oncostatin M), HIF-1α (hypoxia-inducible factor 1 alfa) and NF-κB (nuclear factor kappa B). Its main inhibitor is TGF-β (tumour growth factor beta).[35]

**HGF FUNCTION AND ITS RECEPTOR**

In 1984, HGF was purified for the first time in rat platelets, and 4 years later in humans. It was described as a potent mitogenic factor for mature rat hepatocytes in vitro.[36-39] The HGF/MET pathway has diverse biological and physiological roles in organogenesis, morphogenesis, tissue regeneration and carcinogenesis that have been discovered using conditional knockout of MET in mice.[8] In 1991, a fibroblast-derived factor for epithelial cells, the Scatter factor (SF), involved in increasing cell migration during embryogenesis and tumour progression, together with the human lung fibroblast-derived mitogen, were found to be identical proteins. Both are
encoded by the same chromosome bands as the HGF and are ligands for MET receptor.[40-44] Soon after, a tumour cytotoxic factor derived from fibroblasts, which induces cell death in several cancer types, was also found to be an identical molecule to HGF.[45] These results had important consequences for further studies on the involvement of HGF as a modulator of cellular growth and motility during embryogenesis, tissue regeneration and carcinogenesis. Interestingly, targeted disruption of HGF or MET results in embryonically lethal knockouts with impaired development of the liver and placenta.[46 47] Over-expression, mutations, and amplification of the receptor and/or changes in its kinase activity have been implicated in different types of cancer.[48]

It has been suggested that because of the proximity between HGF and MET on chromosome 7, a polysomy of this chromosome could lead to malignancy secondary to overproduction of both molecules.[49] The one-to-one ligand-receptor relationship makes the HGF/MET axis an attractive target for drug development, either by activation or inhibition of this pathway. Three pharmacologic approaches are currently being developed as inhibitors of MET signalling with promising results: anti-HGF antibodies, anti-MET antibodies and MET kinase inhibitors.[50]

**HGF AND LIVER REGENERATION**

The liver has the ability to regenerate to almost its optimal volume after liver resection.[51] HGF is one of two complete mitogens which induce hepatocyte DNA synthesis and mitosis along with EGFR. Activation of the HGF/MET axis generates a cascade of intracellular signalling for the G1-S progression of hepatocytes. Available evidence has shown that immediately after different liver injuries, such as partial hepatectomy, ischaemia or hepatitis, there is an intense remodelling of the extracellular matrix, with increased activity of proteinases and intense intracellular signalling.[52] The levels of active HGF rise rapidly in the liver secondary to its
production by Kupffer, stellate and sinusoidal endothelial cells, and subsequently
activation by urokinases.[53-55] Since HGF also increases in plasma, an endocrine
mechanism has been suggested. In addition, HGF mRNA and HGF activity have
been found to be markedly higher in other intact organs like the lung, kidney or
spleen after injuries of the liver.[56] Overall, this results in a balanced liver growth
and regeneration.

HGF AND HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the 3rd most common cause of cancer-
related mortality worldwide with a multifactorial aetiology and extensive molecular
and phenotypic heterogeneity.[57] Several signalling pathways have been described
in which the HGF/MET axis may play a crucial role.[58-60] Patients with HCC have
significantly higher serum levels of HGF compared to healthy controls [52 61] and
over-expression of HGF and c-MET has been detected in 33% and 20-48% of HCC
tissues, respectively.[62-66] Up-regulation of MET has also been associated with
tumour migration, vascular invasion, neo-angiogenesis and, therefore, poor patient
outcomes.[67] In contrast, other studies have shown contradictory results with
regards to their impact on survival in HCC patients.[68-73] Nonetheless, clinical trials
using pharmacological inhibitors of this axis for both HCC and other solid
malignancies are currently being performed. These targeted therapies could be a
promising second line option to treat patients with advanced HCC.[60 74 75] A recent
study has demonstrated that the mixed-lineage leukaemia (MLL) protein, in
association with HGF/MET, promotes cell invasion and metastasis in HCC.
Theoretically, the inhibition of this interaction could potentially reduce the incidence
of distant metastasis, although it may not affect the tumour load or its proliferative
capacity.[76]
HGF IN OESOPHAGO-GASTRIC CANCER

Oesophago-gastric cancer (OGC) is the 5th most common malignancy worldwide and the 4th and 5th most common type of cancer death in males & females in UK, respectively. A clear geographical difference has been observed in overall-survival with 70% surviving 5 years in Japan, compared to 25% in Europe, suggesting that the implementation of screening tools might allow earlier detection and treatment of this cancer. OGC have a diverse molecular landscape and exhibit alterations in various different oncogenes and kinase pathways. Activation of the HGF/MET axis promoting tumourigenesis and metastasis in gastric cancer is mainly secondary to MET over-expression and/or amplification, which was observed in 75–90% of cases and in 1.5–20% of the patients, respectively.[77-79] However, mutations of HGF or MET are extremely rare in OGC.[80 81] Different drugs targeting the axis include tyrosine kinase inhibitors (crizotinib, a dual c-MET and ALK inhibitor), and monoclonal antibodies that neutralise HGF (Rilotumumab) or MET (Onartuzumab) are currently being trialled.[82-86]

HGF IN COLORECTAL CANCER

Colorectal cancer is the 3rd most common cancer in men and the 2nd in women worldwide,[87] and around 30% of the patients will develop metastasis even after curative surgery. It has been suggested that the HGF/MET axis is involved in the metastatic progression and potential invasiveness of the cancer cells by regulating the expression of cadherins and extracellular membrane proteases.[88] Besides, MET amplification seems to be a late event in CRC progression. MET amplification is more common in advanced tumour stages [89] and its expression has been found to be higher in metastatic tissue than primary tumour.[90]
Although survival of patients with unresectable metastatic CRC (mCRC) has improved in the last years with the introduction of agents targeting the epidermal growth factor receptor (EGFR), such as cetuximab, its response rate range varies from 10-20%.[91 92] HGF-induced MET activation has been described as a novel mechanism for cetuximab resistance. Dual activation of both EGFR and MET receptors in CRC cells synergistically increases cell proliferation [93]. Cetuximab could inhibit this cell growth by 60-80%. However, addition of HGF to cetuximab-treated cells phosphorylated MET, but not EGFR, restoring cell proliferation. Inhibition of the HGF/MET axis may therefore improve response to EGFR inhibitors in CRC, and combination therapy should be further investigated.[93] A recent study revealed that high levels of serum HGF and epiregulin (EREG) before treatment with anti-EGFR antibodies were associated with poor survival in KRAS wild-type patients with mCRC, suggesting that serum HGF and EREG may be associated with resistance to anti-EGFR treatment. HGF might be a potential biomarker for predicting response and prognosis in dual target therapy with anti-EGFR antibodies and HGF/MET inhibitors.[94]
TAKE HOME POINTS

- HGF is a multi-functional cytokine produced by cells of mesenchymal origin involved in cell proliferation, survival, motility and morphogenesis.
- MET is a tyrosine kinase receptor physiologically expressed on cells of epithelial origin and HGF is its unique binding factor.
- HGF/MET axis plays important roles in embryogenesis, organogenesis, adult tissue regeneration and carcinogenesis.
- Both HGF and MET genes can be over-expressed, potentially mutated, and/or amplified in several types of cancers, including haematological malignancies.
- HGF/MET axis has become the candidate target of numerous therapeutic clinical trials.
FIGURE LEGEND

Figure 1. The HGF/MET axis. Once HGF is activated by a two-cleavage process in the extracellular matrix, it binds to the MET receptor on epithelial cells, promoting its dimerization and auto-phosphorylation of tyrosine residues. The recruitment of adaptor proteins generates different downstream signalling pathways which evoke diverse cellular responses as shown. Key: C-Cbl: Casitas B-lineage Lymphoma; Erk1-2: extracellular-signal-regulated kinases; FAK: focal adhesion kinase; GAB1: Grb2-associated binding protein 1; Grb2: growth factor receptor bound protein 2; Gsk3β: glycogen synthetase kinase 3β; IKK: inhibitor of nuclear factor kappa-B kinase; MEK, mitogen-activated protein kinase/ERK kinase; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor kappa-B; PI3K: phosphatidylinositol 3' kinase; PLCγ: phospholipase C-γ; Stat3: signal transducer and activator of transcription 3. Adapted from [35].
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cisplatin, and capecitabine (ECX) in patients (pts) with locally advanced or metastatic gastric (G) or esophagogastric junction (EGJ) cancer. J Clin Oncol 2012


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