HEPATOCYTE GROWTH FACTOR / c-MET, AN ONCOGENE SYSTEM OF CLINICAL SIGNIFICANCE

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SUMMARY: HGF/c - met oncogene system is a relatively new, subject among the others described so far. But the rapid accumulation of information suggests that it's a very important one, especially from clinical aspects. Hepatocyte growth factor (HGF) is the most potent stimulator of hepatocyte growth and DNA synthesis. The existence of a specific factor that triggers liver regeneration has been defined and purified and termed as HGF. Its well documented effects on cell motility and proliferation attracted special attention on the issue. So, we believe it deserves more attention which could shed light to the mechanism of liver regeneration and oncogenesis. Herein we discussed the basic data regarding HGF/c - met oncogene system.

Key Words: Hepatocyte Growth Factor (HGF), Liver Regeneration, Oncogenesis.

HGF / c-met oncogene system is a relatively new, recent subject among the others described so far. But the rapid accumulation of information suggests that it's a very important one, especially from clinical aspects. So we believe, it deserves special attention for its well-documented effects on cell motility and proliferation.

HGF Oncogene

Hepatocyte growth factor (HGF) is the most potent stimulator of hepatocyte growth and DNA synthesis identified (16, 20, 24, 29). It is known to be the same molecule as the scatter factor, which increases the motility of a variety of cell types (32).

In 1984, the existence of a specific factor that triggered liver regeneration was defined. This factor was isolated in a partially purified form from rat plasma after partial hepatectomy by Nakamura, et al., who termed it "hepatocyte growth factor" (20).

At the same year, two serum factors, called "hepatopoeitin A" and "tumor toxic factor" were reported, and later established to be the same as HGF (15). By 1989, HGF was fully purified, its amino acid sequence determined, and encoding complementary DNA (cDNA) cloned (18, 21). HGF is a heterodimeric heparin-binding protein composed of alfa and beta chains measuring approximately 70 kD and 35 kD respectively (9). The amino acid sequence of human HGF has 31 percent homology with plasminogen. HGF is derived from a 92 kD precursor of 728 amino acids and then processed into two peptide chains (alfa, beta). Its gene is located on chromosome 7, and contains 18 exons and 17 introns. Note that c-met is also located on chromosome 7 and in close approximation.

The aa sequence HGF is a very interesting one, most unusual for a growth factor (17). The alpha chain consists of four kringle domains. Kringles are
double-loop polypeptide structures in which a smaller loop is held together with disulfide bonds within a larger loop. The kringle domains of HGF have substantial sequence similarity to three kringle domains of plasminogen and one kringle domain of prothrombin. The light chain of HGF (beta-chain), on the other hand, has the structure of a pseudoprotease. Strong similarities exist between the beta-chain and serine proteases such as kallikrein and factor XI, although no protease function of HGF has been identified.

Another point is that the mature heterodimer is derived by the cleavage of the single-chain form next to arginine (in position 494) and before valine (position 495). This cleavage region is the same as the one resulting in the activation of plasminogen to plasmin by tissue plasminogen activator (TPA) (17, 23). If this were to be true, it would be quite interesting, given the dramatic increase of TPA in most neoplasms.

In 1985, a fibroblast-derived factor that had major effects on cell motility was described. It was named scatter factor (SF) because it had the ability to scatter tightly growing cell colonies (26). Partial amino acid sequencing revealed more than 90% identity with human HGF (7, 32). The cDNAs encoding HGF and SF are the same and antibodies raised against each factor recognize the other.

HGF has been identified in the plasma of patients with fulminant hepatic failure and the serum levels of HGF of such patients were approximately 30 times higher than in normal subjects (31). In vitro data have shown that HGF is the most potent mitogen for rat and human hepatocytes in serum-free conditions and in the absence of other mitogenic factors. HGF was shown to increase rapidly in plasma before induction of hepatocyte DNA synthesis in the partially hepatectomized or CCL4 treated rat (12). Several papers describe the mitogenic effects of HGF on other cell types, too. HGF stimulates DNA synthesis in renal proximal tubular epithelial cells, melanocytes, keratinocytes, breast carcinoma, and melanoma cell lines... etc (10, 23). HGF does not stimulate DNA synthesis in fibroblasts. Interestingly, some of the hepatoma cell lines tested do not respond to HGF, either.

HGF, now known to be the same as SF, is also potent stimulator of cell motility. A variety of cell types that are important for surgeons such as colon cancer cells, hepatoma cells, and gastric adenoma cells respond in a similar way (9, 25). Several epithelial and endothelial cells scatter on the addition of HGF to the medium. The concentrations causing scattering effects and mitogenic effects do not always coincide, and it has been stated that HGF exercises only scattering effects in some cell lines in the absence of mitogenic activity (17, 27). This is a very important issue, and raises questions about the number of receptors involved in HGF effects and the multiplicity of signal transduction pathways for EGF receptors. Scattering effects are induced only on epithelial and endothelial cells, not on fibroblasts or mesenchymal cells. It should also be mentioned that HGF has both mitogenic and scattering effects on hepatocytes in identical concentrations (20, 24). The scattering effect on hepatocytes appears before and during DNA synthesis. SF can also induce non-transformed cells to behave in vitro as tumor cells, by promoting their invasiveness for collagen matrices.

HGF is present in measurable amounts in normal plasma (15). Many different cell types produce HGF, including Kupffer cells, endothelial cells, i.e. cells of the liver, endothelial cells in the lung, and some malignant cells such as lung and pancreatic carcinoma (30). Among normal tissues; pancreas, small intestine, and submaxillary salivary gland produce and express higher levels of HGF. It still remains to be seen whether EGF protein is actually produced or merely taken up by these cells in which it is localized (17). HGF has so far been found to be produced only by mesenchymal cells but heavily localized in epithelial cells. Therefore, it may be a very important contributor to the trophic interactions, between mesenchymal and paranchymal cells in many tissues. The regulation of HGF production needs further research. For example, injuring is a 10-13 kD circulating protein whose level increases markedly in blood after organ injury and is a potential stimulus for HGF production (13). HGF production and gene expression are triggered by IL-alfa and beta, but inhibited by TGF-beta. Additive effects are seen between HGF and EGF (9, 14).

Liver is the principal organ for initial uptake of HGF. Only a portion of hepatic uptake appears in the bile, and partial hepatocytosis decreases the blood clearance of HGF (1). Low affinity tissues are kidneys, bone, muscle, and skin in decreasing order. Normally HGF undergoes a rapid distribution followed by a relatively slower disposition phase. Both a receptor-mediated and a nonspecific inter-
nalization are involved in this clearance process.

**HGF Receptor - MET Oncogene**

Recent work has suggested that the HGF receptor is the product of the met oncogene, a transmembrane protein endowed with tyrosine kinase activity (22). The protein product of cmet is a 190 kD heterodimer (p190 met) made of a 50 kD subunit (alfa) disulfide-linked to a 145 kD subunit (beta) (3). The molecules is synthesized as a single-chain 170 kD precursor which undergoes co-translational glycosylation. Disulphide arrangement and proteolytic cleavage lead to the mature two-chain 190 kD heterodimer. The alfa chain and the N-terminal portion of the beta chain are exposed at the cell surface. The C-terminal portion of the beta chain is a cytoplasmic kinase domain and phosphorylation sites are involved in regulation of its activity (5). The kinase activity is positively regulated by autophosphorylation on tyrosine, and it is negatively regulated by protein kinase C activation or transient increases in intracellular Ca concentrations (2). Tyrosine kinase transduces all the effects of HGF on motility, growth and morphogenesis. Activation of PKC is thought to inhibit c-met-mediated activities, possibly by the phosphorylation of a specific site on the intracellular domain that interacts with the agonist binding affinity and/or internalization.

High levels of met mRNA have been found in the liver, gastrointestinal tract, thyroid and the kidney (2). Western blot analysis has shown that the levels of met protein (pmet) generally correspond to those of the mRNA (6). However, in the thyroid, where there is a high level of met mRNA, the protein product is barely detectable, suggesting translational or post-translational regulation. Normal or increased levels of met mRNA were consistently found in fresh samples of many carcinomas. In papillary carcinomas of the thyroid, the amount of met protein, almost undetectable in the normal counterpart, was found to be increased more than 100-fold. The tissue distribution of the met/HGF receptor indicates that this molecule is involved in the growth control of many epithelial cells other than hepatocytes and suggests that its increased expression may confer a growth advantage to neoplastic cells (6).

It remains to be seen whether the met protein is the only receptor for HGF. As mentioned before, findings such as the loss of mitogenic effects or the mito-inhibitory and cytotoxic properties at high concentrations, and the apparent dissociation between the scattering and mitogenic effects for some cell lines raise the issue that the met protein may not be the only receptor for HGF (17). On the other hand, the mode of action of pmet is not clearly understood. Better delineation of the signal transduction pathways stimulated by pmet may provide explanations for these disparities and reconcile the existing contradictory data with a single receptor model. Direct studies of binding of HGF on hepatocytes have shown high-affinity binding sites estimated from 1000 to 120000 sites per hepatocyte (33). Lower affinity sites were also detected in higher numbers. The latter may be of importance to the mito-inhibitory effects seen at large HGF concentrations.

For example, met proto-oncogene was found to be amplified and overexpressed, but not mutated in one human gastric tumor cell line, GTL-16. Although after organ resection there is a temporary increase in receptor expression on normal cells, the level of expression returns the normal soon after the insult (28). Normal cells have the ability to control their response to HGF by reducing receptor expression, whereas malignant cells may consistently overexpress the receptor and may over-react to HGF (9). Another example is that; met constitutive activity in a colon carcinoma cell line was thought to be due to incomplete processing because of a defect in the cleavage of the precursor molecule mentioned above (19). The 190 kD precursor appears in the plasma membrane. The precursor is itself thought to be active without HGF binding, and these cells have increased motility in the absence of exogenous HGF.

**The clinical deduction**

Kaneko et al. showed in patients with liver metastases who had higher levels of circulating HGF than normal subjects, that these levels were raised further after resection of metastatic foci in the liver (11). Even routine surgical operations such as cholecystectomy, colectomy, and splenectomy may induce specific increases in blood HGF levels. By inducing epithelial cell proliferation and motility, can also stimulate neovascularization in vivo (4). Thus, cancer cells expressing HGF receptors will become more invasive and may form distant metastases when exposed to excess levels of HGF generated as a result of certain insults such as liver or kidney resection, or even the excision of the tumor itself.
Therefore, any invasive procedure, in addition to suppressing NK cell activity, may also induce HGF production and provoke tumor growth and metastasis. In melanoma lines for instance, phosphorylation of tyrosine residues on c-met was found to induce a cascade of tyrosine phosphorylations on several other proteins and activation of microtubule-associated protein (MAP) kinase. HGF may therefore promote dispersion of melonocytes from early stages of primary melanomas to ectopic sites (8).

In conclusion, the HGF-met oncogene system is a vast area of intense research. Many normal and tumor cell lines are stimulated with respect to cell motility and DNA synthesis by HGF. The inconsistency of responses of certain cell types and certain dose-dependent variations in the relevant HGF effects necessitates that the signal transduction pathways involved in this system be defined in a more precise manner. Nevertheless, the well-documented scattering effect of HGF must be taken into account before any invasive procedure is intended. Competitive antagonists of HGF (recently reported) or c-met antisense oligonucleotides are examples of the future potential lines of approach, especially before any traumatic intervention to the tumor is carried out.

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