The role of HGF - c-met system in tumor progression

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I. Introduction

In Hungary as well as in the rest of world cancerous diseases are leading diseases and cause of death. The main cause of death is formation of metastatic cancer. For this reason it is highly important to understand and know molecular mechanism of tumor progression. This knowledge is needed to extend the therapeutic possibilities.

The matter of this study is the role of hepatocyte growth factor and its receptor, the c-met in the tumor progression, focusing on colorectal tumors.

The metastasis of human tumors is a highly selective process as in case of different type of tumors different organs are involved. The most frequent site of metastasis is the liver. Liver metastasis is typical for different human tumors but specially for colorectal cancers. It is known to be determined by anatomical factors such as the portal vein collects the veins of colorectum. But nowadays this opinion has been
changed. It turned out that tumor cell and liver specific factors can play a crucial role in this process (1, 2, 3). Not only the liver but bones are involved in the progression of colorectal carcinomas, too. Nevertheless the macroscopic metastases are formed in liver (4).

In the development of an organ metastasis one of the main points are the present growth factors in the given organ. In the case of liver it could be the HGF. The HGF is produced by Ito cells in liver (5). It has a physiological role in the regeneration after liver damage (6). The most part of HGF is localised in periportal areas in the liver. The mitogenic signal starts from periportal areas after liver injury (7). Plasma level of HGF in human is lower than 0.5 ng/ml (8), in rat is 20 ng/ml (9).

The receptor of HGF is the c-met oncoprotein (10). Its gene is localised 7th chromosome q21-31 in human. C-met is a heterodimeric protein containing an 50 kDa \( \gamma \) chain and an 145 kDa \( \alpha \) chain. The \( \gamma \) chain is built from extracellular, transmembrane, and intracellular catalytic domain (11, 12, 13, 14). In human epithelial cells c-met can be expressed in different forms by alternative splicing. This results 200, 170, 65 kDa forms.
Last one missing extracellular domain has src-like tyrosine kinase activity (15).

Binding of HGF to cell surface heparane sulphate proteoglycans enhances the signal transduction on c-met, according to the literature (16). Earlier studies show that cell surface proteoglycans play an important role in metastasis formation of some rodent and human tumors (17, 18). These data suggest the play of cell surface HSPGs in metastasis.

The interaction between HGF and heparin is mediated by positively charged residues (Lys, Arg, His) of cytokine and the negatively charged sugar components (sulphate or carboxyl chains) of glycosaminoglycans of proteoglycans (19, 20, 21). The minimal condition of the protein – GAG interaction is an oligosaccharide of 5-6 unit and a basic peptide containing 5-6 residue (22, 23).

In the tumor progression growing is a repeating step: both the prime and metastatic tumor have to grow. Its condition is not only the appropriate biomolecular circumstance (growth factors, receptors), but tumor cells require sufficient nutrition and oxygenation. In absence
of vessels the size of tumor could be limited by diffusion of oxygen in tissues. But tumors can induce angiogenesis.

The tumor cells are able to induce angiogenesis using physiological pathways (24, 25). The absence of expression of thrombospondin, a physiological inhibitor of angiogenesis, could be the consequence of amplification of oncogenes and absence of wild type p53 expression. In this way the equilibrium between angiogenic and antiangiogenic factors is upset (26).

There are different ways for tumor induced angiogenesis:

1. The peritumoral vessels grow into the tumor.
2. The net of the vessels is continuously growing on edge surface of tumor and stroma. Later these vessels are overgrown by the tumor (27).
3. In the case of vessel cooption the tumor overgrow the existing host vessel (28), then the endothelial cells are degraded because of dominance of antiangiogenic factors (29). This can result sinuses edged by tumor cells.
4. In the case of vascular mimicry sinuses are formed by tumor cells expressing endothel specific genes (30).
II. Aims

Based on foregoing we wanted to answer next questions:

1. Does the HGF as the one of the local growth factors of liver play role in the metastasis of colorectal carcinomas? Namely, does HGF influence the biological behaviour (proliferation, migration) of human colorectal cancers?

2. Does the HGF and c-met influence neoangigenesis in the case of human colorectal cancers?

3. Have the heparin binding sequences of HGF got any tumor biologically significant activity?

4. Have the heparin binding sequences of HGF got any effect on angiogenesis?
III. Materials and methods

The biological effects of HGF and the basic peptides were tested in in vitro proliferation assay and scatter assay. Their effect was tested on growth of subcutaneously transplanted HT25 human colorectal tumor or on growth and liver metastases of HT25, HT168-M1/9, 3LL-HH tumors inoculated into spleen, respectively.

Cell lines: HT29, HT25, WiDr human colorectal carcinomas, HT168-M1 human melanoma, KS-IMM human Kaposi sarcoma, HBE primer culture of cerebellar microvascular endothelial cells.

Patients: eighty six colorectal patients of National Institute of Oncology were included in the study (46 Duke's B, 40 Duke's C). Each were analysed by western blot. Twenty five tumors (11 Duke's B, 14 Duke's C) were analysed by immunohistochemistry.

The angiogenic effects of investigated materials were tested on in ovo chorioallantois membrane model and on tumor induced angiogenesis of subcutaneously transplanted HT25 tumor.
Expression of heparane sulphate proteoglycans on human colorectal carcinoma cell lines was tested by flow cytometry.

The expression of c-met oncoprotein was detected by methods of molecular biology such as RT PCR, western blot analysis on adenocarcinoma cell lines or human tumors, flow cytometry or by morphological methods such as confocal laser scanning microscopy on adherent adenocarcinoma cell lines, immunohistochemistry on surgical specimens of human colon cancers.
IV. Results

Three human colon cancer cell lines with different metastatic potential was studied. Two of them (HT25, WiDr) have given metastasis in SCID mouse spleen-liver model but third one (HT29) not.

C-met expression of these cell lines was determined. Our data from RT-PCR, Western blot, flow cytometry and immunocytochemistry have shown that total chain of c-met is expressed on detectable level only in the two metastatic cell line.

The biological effect of HGF was tested on the 3 colon cancer cell lines. The proliferative capacity of tumor cells was not influenced by HGF. On the other hand HGF enhanced the motility of the 2 metastatic cell line but not of non metastatic one (based on scater activity).

C-met expression was studied on surgical specimens of colon cancers (Duke’s B and Duke’s C) by Western blot analysis and immunohistochemistry. The 40% of Duke’s B cases was negative by
immunohistochemistry, but the strong staining dominated in Duke’s C group. It was proved by densitometrically evaluated Western blot of tumor tissues that the expression of c-met in Duke’s C cases is significantly higher than in Duke’s B cases (IV).

HGF is a member of family of heparin binding cytokines. Its basic residue rich sequences are responsible for this feature. For this reason the biological effect of basic peptide sections of HGF α chain (HGP peptides) and artificial basic hexapeptides (BP peptides) was studied on colorectal cancer cell lines, too. The next peptides were studied: HGP1=HHRGK$_{645-649}$, HGP2=RYRNKH$_{512-516}$, BP3=KHKKKH, BP4=KRKRKR, BP5=HRHRHR, BP6=HRKHRK (József Tímár’s patent).

The proliferation of HT25 human colorectal cell line and M1/9 human melanoma cell line was inhibited only by high doses (100-1000 ng/ml) of HGP1 and HGP2 peptides (V).

The subcutaneous growth (0.5, 0.05 mg/kg/day, peritumorally) and liver colonisation (500 mg/kg/day
i.p.) in SCID mouse was inhibited by HGP1 peptide. The liver colonisation of 3LL-HH murine lung carcinoma cell line and M1/9 cell line in spleen-liver model in SCID mouse was inhibited by HGP1 peptide (500 ng/animal i.v.), too (V).

The proliferation of HT25 human colorectal cell line and M1/9 human melanoma cell line was inhibited by high doses (100-1000 ng/ml) of BP3, BP4, BP5, BP6 peptides (V). The liver colonisation of 3LL-HH murine lung carcinoma cell line in spleen-liver model was inhibited by BP4 peptide (50, 500 ng/animal, i.v.) (V).

It is known that heparin binding peptides of some angiogenic citokines have antiangiogenic effect that is why the angiogenic effect of HGP and BP peptides was studied, too.

The angiogenesis in chick chorioallantoic membrane (CAM) model was inhibited by HGP1, HGP2 and BP4 peptides (10-100 ?g/egg). The proliferation of human cerebellar vascular endothelial cells was inhibited by these peptides, but they did not influenced the
proliferation of KS-IMM Kaposi’s sarcoma cells (100-1000 ng/ml) (I).

The HGP1 peptide was tested in tumor induced angiogenesis model. The neoangiogenesis induced by subcutaneously transplanted HT25 human colorectal carcinoma was studied in SCID mice by immunocytochemistry and morphometry (50 ?g/animal peritumorally). The local administration of HGP1 could not prevent the tumor induced angiogenesis, but the perimeter of formed vessels significantly was decreased. It means it acts in morphogenic phase of angiogenesis but not in the proliferative phase.
V. **New establishments of the dissertation**

1. The human recombinant HGF increases the motility of human colorectal carcinoma cell lines depending on their metastatic potential, while their proliferative potential not. Differences in c-met expression could be in the background of this phenomenon. The fact that higher c-met expression was detectable in metastatic Duke’s C colorectal cancer than in non-metastatic Duke’s ones proves the clinical importance of HGF/c-met system.

2. The human recombinant HGF could inhibit angiogenesis in chick chorioallantois membrane model.

3. Heparin binding basic sequences of HGF ? chain could inhibit the proliferation of HT25 human colorectal tumor cell line and M1/9 human melanoma cell line. One of them (HGP1) could inhibit the in vivo liver metastasis of HT25, M1/9 cell lines and 3LL-HH rodent lung carcinoma cell line.

4. Heparin binding basic sequences of HGF ? chain could inhibit angiogenesis in chick chorioallantois
membrane model. The morphogenic phase of angiogenesis was inhibited by HGP1 peptide.

To summarise the above facts we can say:

A. The HGF – c-met system could play a role in the metastasis of human colorectal cancers. For this reason the HGF – c-met system can be a considerable therapeutic point of attack.

B. The small basic peptides of HGF have antiproliferative, antimeta staic, antiangiogenic effect showing that small basic peptides can be the start point of new anticancer drugs.

VI. Own publications on topic if dissertation

Publications in English


Publications in Hungarian


Chapters of book:

Abstracts:


XII. Fazekas K., Paku S., Tímár J.: C-met onkoprotein (HGF receptor) szerepe humán kolorektális tumorok biológiai viselkedésében. Magyar Onkológia 43(2):254, 1999.

VII. Literature


15. Rodrigues NA, Naujokas MA, Park M. Alternative splicing generates isoforms of the met receptor