

# Gene expression of hepatocyte growth factor and its receptor in HCC and nontumorous liver tissues

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**Subject headings** liver neoplasm; hepatocyte growth factor receptor; hepatocyte growth factor; gene expression

## Abstract

**AIM** To study the changes of gene expression of hepatocyte growth factor (HGF) and hepatocyte growth factor receptor (HGFr) in hepatocellular carcinoma (HCC) tissue and nontumorous liver tissue and the relationship between these changes and the biological behavior of the tumor.

**METHODS** Gene expression of HGF and HGFr in 26 cases of HCC tissue and their adjacent nontumorous liver tissues was determined with digoxigenin-labeled DNA probes.

**RESULTS** Positive expression of HGF in HCC tissue was similar to that in the adjacent nontumorous liver tissue, but positive rate of HGF expression was lower than HGFr gene expression. However, HGFr expression was higher in the metastatic cases than in those without metastasis. It was found that HGFr was overexpressed in HCC tissue as well as in the adjacent nontumorous liver tissue.

**CONCLUSION** There seems to be a close relationship between overexpression of HGFr gene and tumor metastasis, and the HGF and HGFr system plays an important role in regulating tumor growth and metastasis.

## INTRODUCTION

Gene mutation is the pathogenetic basis and essence of canceration induced by various carcinogenic factors. Mutation of oncogene and anti-oncogene, alteration of growth factor and growth factor receptor as well as instability of chromosome are the essential causes of tumor genesis. Using the hepatocyte growth factor (HGF) and hepatocyte growth factor receptor (HGFr) gene (c-met) DNA probe, we conducted an auto-control study on primary liver cancer tissues and their adjacent nontumorous tissues in 26 patients suffering from hepatocellular carcinoma (HCC) in an attempt to observe expression of gene products at a transcription level and thus to explore the role and significance of these products in the development and growth of liver cancer.

## MATERIALS AND METHODS

### *Specimens*

Tissue specimens used in the present study were sampled from 26 patients with pathologically confirmed hepatocellular carcinoma and the nontumorous liver tissue (2 cm away from the carcinoma), including 22 males and 4 females ranging in age from 34 to 63 years with a mean of 48.5 years. The fresh tissue was surgically excised under aseptic conditions and stored in liquid nitrogen.

### *RNA extraction*<sup>[1]</sup>

A tissue mass of 0.5 g with addition of 4 mol/L-guanidinium thiocyanate, 25 mmol/L-sodium citrate, 5 g/L-Sarcosyl and 5 mL of 0.1 mol/L- $\beta$ -mercaptoethanol solution was ultracentrifuged in an ice bathed centrifuge tube for 1 min-2 min, followed by addition of 0.5 mL of 2 mol/L-NaAc (pH 4.0), 5 mL-saturated phenol and 1 mL chloroform-amylen alcohol (49:1), which were mixed up thoroughly by oscillation for 5 min and ice bathed for 15 min. The stock solution was centrifuged at 4°C at 9 200rpm for 20 min. The supernatant was mixed with isopropanol thoroughly, centrifuged at 9 200rpm for 20 min, precipitated at -20°C for 2 h, and then centrifuged at 2 200rpm at 4°C to discard the supernatant. The precipitate was washed with 75% alcohol and vacuum dried. The RNA was dissolved in 0.5 mL-water pretreated with diethylpyrocarbonate (DEPC) and then stored at -70°C for use.

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### Preparation and labeling of the probe

**Source of the probe** The HGF probe plasmid was kindly donated by the American Gene Engineering Co., and the HGFr plasmid (c-met plasmid) given by the Gene Bank of the Japanese Cancer Research Resources.

**Preparation and labeling of the probe** The probe was prepared and labeled as described<sup>[2]</sup>. The HGF plasmid was enzyme-cut with -EcoR I-EcoR-V to obtain a 1.4 kb segment as the probe. The c-met plasmid was enzyme-cut with -EcoR-I-Sal-I to obtain a 1.6 kb segment as the probe. Labeling was performed by the digoxin random primer method.

### RNA dot blot hybridization

**Preparation of the hybridization membrane**  $\mu$ LRNA was mixed with the following solutions: 20  $\mu$ L 1L/L- formamide, 7  $\mu$ L 370 mL/L-formaldehyde, 2  $\mu$ L 20 $\times$ SSC at 68 $^{\circ}$ C, which was cooled in ice bath promptly at 15 min. The denatured RNA was spotted on to the nitro-cellulose membrane, with 10  $\mu$ L-at each spot (the membrane was wet with water and then soaked in 20 $\times$ SSC for 1h), dried in a vacuum drying oven at 80 $^{\circ}$ C for 2 h, and then sealed in a plastic bag for use.

**Hybridization** Hybridization was conducted according to the instructions of the digoxin DNA labeling test kit.

### In situ molecular hybridization

**Treatment of the slide** The slide was soaked in sulfuric acid overnight, washed, dried at 180 $^{\circ}$ C for 3 h, and then coated with gelatin to which 0.1% DEPC was added to inhibit RNase activity.

**Preparation of frozen sections** Frozen sections of 6  $\mu$ m in thickness dried with cool air flow were fixed with 4% paraformaldehyde buffer solution for 10 min, gradient dehydrated with ethanol, and stored at -70 $^{\circ}$ C for use.

**Procedures of hybridization in situ** This was done according to the instructions of the digoxin DNA labeling test kit.

### Statistical treatment

Percentage of the specimens was compared by  $\chi^2$  test.

## RESULTS

### Results of RNA dot blot hybridization

The results showed that HGF was expressed both in the liver cancers (5/26) and in the nontumorous liver tissues (6/26); the positive rates of expression were low, but they showed no significant difference

between the two groups; c-met expression was relatively high in the liver cancers and the nontumorous liver tissues, the former being higher than the latter (17/26 vs 8/26), showing significant difference ( $P < 0.05$ ).

### Results of hybridization in situ (ISH)

The results of hybridization in situ were classified into four grades (Table 1): negative, weak, moderate and strong. HGF gene expression was not high either in the liver cancers or in the nontumorous liver tissues, while c-met gene expression was high, similar to the result of dot blot hybridization. So far as distribution was concerned, weak and moderate grades dominated HGF gene expression while moderate and strong grades dominated c-met gene expression.

Table 1 The results of RNA ISH of HCC tissue and nontumorous liver tissue

Group	n	HGF expression				c-met expression			
		N	W	M	S	N	W	M	S
HCC	26	20	2	3	1	9	1	6	10
Nontumorous	26	18	3	3	2	13	4	6	3

N: Negative; W: Weak(10%-20%); M: Moderate(40%-60%); S: Strong (70%-80%).

### Relationship between c-met gene expression in cancer tissues and tumor metastasis

Comparative study showed that c-met gene expression was much higher in the group with associated metastasis than that in the group without associated metastasis (15/18 vs 2/8), showing significant difference between the two groups ( $P < 0.01$ ).

## DISCUSSION

### Traits and significance of HGF and HGFr expression in liver cancer and nontumorous liver tissue

HGF has a wide spectrum of biological activity. Recent studies also showed that HGF could inhibit growth of HCC cells as well as AFP gene expression of HCC<sup>[3]</sup>. HGFr is the product of c-met cancer gene, and c-met mRNA has been found to be over-expressed in various tumor tissues<sup>[4]</sup>. No report has been seen about the law of change in HGF and HGFr gene expression in liver cancer. The results of our study showed that although HGF expression was observed both in the liver cancer and the nontumorous liver tissues, its positive rate was much lower than c-met gene expression; c-met gene expression was high both in the liver cancer and in the nontumorous liver tissue, and expression in the former was higher than that in the latter. The mode of c-met expression here was similar to that in other tumor tissues. There was no difference in HGF ex-

pression in the liver cancer and nontumorous liver tissues. In the presence of high expression of c-met gene, sensitivity of malignant tumor cells to HGF was increased; only small amounts of HGF would trigger pronounced response, resulting in extension and metastasis of the tumor. Therefore, determination of c-met gene expression may help assess malignancy and prognosis of a tumor.

### ***C-met gene overexpression and tumor metastasis***

Studies have shown that c-met mRNA concentration is very low or undetectable in normal tissue, whereas it has high expression in the corresponding cancerous tissues. Once a cell with negative HGF is infected, its receptors may be irritated by HGF and activate the cells, which then invade the basement membrane. Malignant cells with high concentration of HGFr have more sensitive and stronger response to HGF<sup>[5]</sup>. Studies reveal that cancerous cells have a defect of c-met protein retro-transcription and processing. The production of this protein involves synthesis of 190kb primary gene, cleavage of  $\alpha$  and  $\beta$  sub-units of c-met and formation of mature HGFr which all need the integration of HGF<sup>[6]</sup>. Some cancerous cells have defective cleavage. As the primary gene itself is active, the cancerous cells would be out of HGF control even if the cells do not split. Cell activity would be increased even in the absence of exogenous HGF. The expression of HGFr on the malignant cell is different from that on the normal cell. Although HGFr expression of normal cells may be increased temporarily after resection of the liver or the kidney, it restores to normal soon after inju-

ry. This is because normal cells and organs are capable of lowering receptor expression and regulating their response to HGF, whereas persistent overexpression of HGFr in malignant tumor cells leads to its excessive reaction with HGF, providing malignant tumor cells with kinetic and aggressive traits<sup>[7]</sup>. The present study revealed that there was a tendency of overexpression of c-met gene in patients with metastatic malignancy, over expression of c-met gene was closely correlated with tumor metastasis and infiltration, and that HGF and its receptor system play an important role in regulating growth and metastasis of liver tumors.

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