The clinical value of hepatocyte growth factor and its receptor—c-met for liver cancer patients with hepatectomy

F. Wu a, L. Wu b, S. Zheng c,∗, W. Ding d, L. Teng a, Z. Wang d, Z. Ma b, W. Zhao a

a Department of Surgical Oncology, First Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, Zhejiang Province, China
b Institute of Infectious Diseases, First Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, Zhejiang Province, China
c Department of Hepatobiliary Surgery, First Affiliated Hospital, Zhejiang University College of Medicine, 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China
d Department of Pathology, First Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, Zhejiang Province, China

Received 25 August 2005; accepted 6 March 2006

Available online 19 April 2006

Abstract

Background. To study the dynamic change of hepatocyte growth factor after hepatectomy in patients with primary liver cancer, and to analyse the prognostic value of hepatocyte growth factor and c-met for these patients.

Methods. Thirty-one consecutive patients undergoing partial hepatectomy for liver cancer were studied. Serum hepatocyte growth factor level was determined by using enzyme-linked immunosorbent assay kit before and after operation, respectively. C-met protein and mRNA expressions in cancerous and paracancerous tissues were examined by immunohistochemical and RT-PCR methods, respectively. The correlations between clinical-pathologic parameters and the expressions of hepatocyte growth factor in serum and c-met in cancerous tissues were analysed, respectively.

Results. Liver cancer patients had a significantly higher level of serum hepatocyte growth factor than normal controls (1.0424 ± 0.498 ng/ml versus 0.685 ± 0.115 ng/ml, p = 0.008). Serum hepatocyte growth factor level was positively affected by tumour size, node cirrhosis, portal vein tumour thrombi, cholangiocarcinoma (including combined hepatocellular carcinoma), poorly differentiated hepatocellular carcinoma and tumour recurrence or metastases. After hepatectomy, serum hepatocyte growth factor level peaked on the third postoperative day, and then declined, but did not return to normal level on the postoperative day 10. From the preoperative day to postoperative day 10, the level of serum hepatocyte growth factor had a decrease of percent (85.33 ± 10.2%) in the group with large tumours (>5 cm), but an elevation of percent (121.9 ± 10.3%) in the group with small tumours (≤5 cm). From the preoperative day to postoperative day 3, the level of serum hepatocyte growth factor had a higher elevation in the group with major resection than in the group with local resection (p = 0.016). Moderately or strongly positive expression of c-met protein was observed in 27 cancerous regions (27/31), and only in 5 paracancerous regions. The intensive expression of c-met mRNA was 100% (31/31) detectable in the cancerous tissues, but only 22.6% (7/31) in the paracancerous tissues. C-met protein expression in cancerous tissues was correlated with portal vein tumour thrombi, cholangiocarcinoma and tumour recurrence or metastases, and the expression in paracancerous tissues was correlated with node cirrhosis. No significant correlation was observed between the hepatocyte growth factor in serum and c-met in cancerous tissues.

Conclusion. The over-expressions of the hepatocyte growth factor and c-met indicates an adverse prognosis for patients with liver cancer. The sustained high level of serum hepatocyte growth factor after hepatectomy may be a factor related to early tumour recurrence and metastasis. Liver regeneration may be a main factor leading to high level of serum hepatocyte growth factor in early postoperative stage.

© 2006 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.

Keywords: Carcinoma; C-met; Hepatectomy; Hepatocyte growth factor (HGF); Liver; Liver regeneration

1. Introduction

Intrahepatic recurrence and metastasis are two main factors that will affect the surgical outcome in patients with liver
cancer. Hepatocyte growth factor (HGF) and its receptor c-met play an important role in tumour migration and metastasis [1]. HGF was first purified from human and rat platelets on the basis of its ability to stimulate mitogenesis of rat hepatocytes. The c-met protein is a tyrosine kinase receptor with an extracellular 50-kDa α-subunit and a transmembrane 145-kDa β-subunit. HGF stimulates proliferation, migration and morphogenesis of epithelial cells by specific binding to its receptor c-met [2,3]. Hepatocytes are highly differentiated cells that rarely divide under physiological conditions; however, they will have proliferative capacity to adapt to varying metabolic demands. Hepatocytes may be induced to proliferate following toxic damage and surgical resection [4–6]. Hepatocyte growth factor (HGF) and its receptor c-met [2,3].

The rapid metabolic adaptations occur in the early stage following partial hepatectomy or injury. HGF is a primary agent promoting the proliferation of mature hepatocytes [5,8]. Following partial hepatectomy, there is a dramatic increase of HGF levels in serum prior to the onset of DNA synthesis by the liver remnant [6]. The proliferative process in liver lasts until it reaches a level to which most of the original mass is recovered.

2. Materials and methods

2.1. Patients

Thirty-one patients underwent surgical resection from January 2004 to January 2005 in our hospital. The mean age of these patients was 51 (range 36–72) years, including 25 males and 6 females. Pathologically, these lesions matched the standards of primary liver cancer. The classification of these tumour tissues were as follows: hepatocellular carcinoma (HCC) (in 24), cholangiocarcinoma (cholangiocellular carcinoma, CC) (in 2) and HCC–CC (combined HCC) (in 5). According to Edmondson grade, of the 24 HCC, tumours were found well and moderately differentiated in 17, while poorly differentiated in the other 7.

Surgical methods were as follows: major resection for 18 patients (left hemi-hepatectomy for 3 patients and lobectomy for 15 patients), and local resection (including segmentectomy) for 13 patients. These patients had been followed up for 6–18 months. Six patients had intrahepatic tumour recurrence and two patients had distant metastasis including one lung and one-bone metastases.

The clinical-pathologic factors were as follows: major resection for 18 patients (left hemi-hepatectomy for 3 patients and lobectomy for 15 patients), and local resection (including segmentectomy) for 13 patients. These patients had been followed up for 6–18 months. Six patients had intrahepatic tumour recurrence and two patients had distant metastasis including one lung and one-bone metastases.

The clinical-pathologic factors were as follows: preoperative α-fetoprotein (AFP) level, cirrhosis status, tumour size (>5 cm versus ≤5 cm), portal vein tumour thrombi (PVTT), pathological classification and postoperative tumour recurrence or metastasis. The correlations between the factors mentioned above and the HGF level in serum and c-met expression in cancerous tissues were analysed, respectively.

2.2. Sample collection

Cancerous and the paracancerous tissues (2 cm away from the carcinoma) were obtained through hepatectomy. The specimens were immediately frozen in liquid nitrogen and then kept at a temperature of −80 °C until being examined. Blood samples of all the patients were collected 1 day before operation, and 3, 7 and 10 days after operation, respectively. Blood samples of 20 healthy adults were collected as controls. The serum was separated in 30 min after coagulation at room temperature and was centrifuged at 3000 rpm for 15 min. The separated sera were stored at a temperature of −80 °C until being assayed.

2.3. Reagent

The human HGF immunoassay kit was purchased from R&D Systems Inc. Rabbit anti-human HGF R (c-met) antibody was purchased from Beijing Zhongshan Gold Bridge Biotechnology Co. Ltd., China. Ultra-sensitive TM S-P kit was purchased from Fuzhou Maixin Biotechnology Development Company, China. TRIZOL™ and SuperScript kit were got from Invitrogen Company. Primers for c-met and β-actin were synthesised by Shanghai Sangon Biological Engineering & Technology and Service Co. Ltd., China.

2.4. Serum HGF assay

Serum HGF was determined by Quantikine enzyme-linked immunosorbent assay (ELISA) kit. The HGF concentration in a sample was determined by computer software-generated interpolation (Microsoft Origin software) under the standard curve. Standard curve was generated and plotted by a log–log linear regression. The minimum detectable dose of HGF was 40 pg/ml.

2.5. Detection of c-met protein by immunohistochemical analysis

Frozen tissues were ordinarily fixed with formalin and embedded with paraffin. Tissue sections were deparaf-finised in xylene and dehydrated in a series of ethanol solutions. The section was treated with blocking solution, primary antibody (polyclonal rabbit anti-c-met antibody 1:100) and horseradish peroxidase-conjugated secondary antibody (LSAB). Bound peroxidase was visualised by 3,3′-diaminobenzidine (DAB) as chromogen. In negative controls, PBS replaced the primary antibody. C-met positive expression was the brown particles in the cytoplasm and cell membrane in cancerous and paracancerous tissues. Ten areas were randomly selected and counted at a magnification of 200. C-met staining was evaluated semi-quantitatively on the basis of the percentage of positive cells, and classified as follows: intensive positive (+++) when positive cells comprised more than 50% of the total cells; moderately
positive (+++) when positive cells comprised 16–50%; weakly positive (+) when positive cells comprised 10–15%; and negative (−) when positive cells comprised less than 10% (Fig. 1).

Fig. 1. C-met expression by immunohistochemical analysis (magnification of 200). Photo 1: paracancerous tissue; photo 2: HCC (Edmondson grade 3); and photo 3: CC (poor differentiation).

Fig. 2. C-met mRNA expression by RT-PCR. M: DNA marker; 1, 3: HCC (grade 2); 2: HCC (grade 3); 4: cholangiocarcinoma (grades 2 and 3); and 5, 6, 7, 8: the paracancerous tissues of cases 1, 2, 3, 4, respectively.

2.6. Detection of c-met mRNA by RT-PCR

The frozen tissues were homogenised completely in 1 ml of TRIzole™ (Invitrogen) and shacked for 15–30 s with 0.2 ml of chloroform. Total RNA was extracted by SuperScript kit (Invitrogen). A 370-bp fragment of the c-met cDNA was amplified with the following primers: 5′-ACGTGCAGTGAAGTGGATG-3′ (sense) and 5′-GAA-GGATACGGAGCGACACA-3′ (antisense). β-Actin gene was used as control. PCR sequence of primer for β-actin was as follows: sense, 5′-CGC TGC GCT GGT CGT CGA CA-3′ and antisense, 5′-GTA ACG CAC GAT TTC CCG CT-3′ (619 bp product). Thirty-five cycles were performed, each consisting of 95 °C, 45 s; 58 °C, 1 min. There was a time delay of 10 min at 72 °C. The reaction products were visualised by 15 g/l agarose gel electrophoresis (Fig. 2).

2.7. Statistical analysis

All the data were analysed by using SPSS 10.0 for Windows. ANOVA, Student’s t, non-parameter and chi-square tests were used to compare the differences between corresponding groups. \( p < 0.05 \) was regarded as statistically significant.

3. Results

3.1. Evaluation of serum HGF concentrations

Compared with normal controls, the liver cancer patients had a significantly higher preoperative concentration of serum HGF (1.0424 ± 0.498 ng/ml versus 0.685 ± 0.115 ng/ml, \( p = 0.008 \)). These concentrations peaked on the third postoperative day (POD), and then declined to 1.028 ± 0.42 ng/ml and 0.946 ± 0.362 ng/ml on the seventh and tenth POD, respectively. However, the HGF concentration on the tenth POD was still significantly higher than the normal controls. Except for the group of the third POD, the HGF concentrations had no significant difference among the other groups (Table 1). Preoperative HGF concentrations were positively affected by larger tumour (>5 cm), node cirrhosis, PVTT, pathology classification (cholangiocarcinoma and poorly differentiated HCC) and postoperative tumour recurrence (Table 2). From the preoperative day to the third POD, the concentration of serum
Table 1
Serum HGF level before and after operation

<table>
<thead>
<tr>
<th>Group</th>
<th>HGF (ng/ml)</th>
<th>S.D.</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before operation</td>
<td>1.0424</td>
<td>0.498</td>
<td>0.089</td>
</tr>
<tr>
<td>POD 3</td>
<td>1.422 a</td>
<td>0.834</td>
<td>0.15</td>
</tr>
<tr>
<td>POD 7</td>
<td>1.028</td>
<td>0.432</td>
<td>0.078</td>
</tr>
<tr>
<td>POD 10</td>
<td>0.946</td>
<td>0.362</td>
<td>0.065</td>
</tr>
<tr>
<td>Control</td>
<td>0.685 a</td>
<td>0.115</td>
<td>0.02</td>
</tr>
</tbody>
</table>

HGF normal limit: 0.46–0.91 ng/ml.

a Compared with other groups, p < 0.05.

HGF had a higher elevation in the group with major resection than in the group with local resection (p = 0.016); however, no significant change was found on the tenth POD. From the preoperative day to the tenth POD, the concentration of serum HGF had a decrease of percent (85.33 ± 10.2%) in the group with large tumour (>5 cm), but an elevation of percent (121.9 ± 10.3%) in the group with small tumour (≤5 cm), the two groups had a significant difference (Table 3).

3.2. Expressions of c-met protein and mRNA in cancerous and paracancerous tissues

The results of c-met protein expression were classified into three grades, which were as follows: negative or weak positive, moderate positive and strong (intensive) positive. The moderately or strongly positive expression of c-met protein was higher in cancerous tissues (27/31) than in paracancerous tissues (5/31). Negative of c-met protein was detected in 17, and the weak positive expression was detected in nine paracancerous tissues (Tables 4 and 5). The c-met protein was found only in hepatocytes and tumour cell. The intensive expression of c-met mRNA was 100% (31/31) detectable in the cancerous tissues, but only 22.6% (7/31) in the paracancerous tissues. The expression intensity of c-met protein in cancerous tissues was correlated with PVTT,

Table 2
The correlations between clinical-pathologic factors and the expressions of HGF in serum and c-met in cancerous tissues

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>HGF (ng/ml)</th>
<th>S.E.</th>
<th>p</th>
<th>C-met protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Node cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>1.339</td>
<td>0.127</td>
<td>0.008</td>
<td>3  12  5</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>0.859</td>
<td>0.104</td>
<td>1</td>
<td>4  6  0.263</td>
</tr>
<tr>
<td>2. Preoperative AFP (ug/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20</td>
<td>14</td>
<td>1.108</td>
<td>0.139</td>
<td>2</td>
<td>5  6  0.758</td>
</tr>
<tr>
<td>&gt;20–400</td>
<td>8</td>
<td>0.714</td>
<td>0.127</td>
<td>0.255</td>
<td>1  6  2</td>
</tr>
<tr>
<td>&gt;400</td>
<td>9</td>
<td>1.278</td>
<td>0.277</td>
<td>1</td>
<td>5  3  0.079</td>
</tr>
<tr>
<td>3. Tumour size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 cm</td>
<td>18</td>
<td>0.782</td>
<td>0.079</td>
<td>0</td>
<td>4  9  0.079</td>
</tr>
<tr>
<td>&gt;5 cm</td>
<td>13</td>
<td>1.371</td>
<td>0.136</td>
<td>0.001</td>
<td>0  7  6</td>
</tr>
<tr>
<td>4. Pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCC Edmondson I and II</td>
<td>17</td>
<td>0.787</td>
<td>0.09</td>
<td>0.028</td>
<td>3  12  2</td>
</tr>
<tr>
<td>HCC Edmondson III and IV</td>
<td>7</td>
<td>1.341</td>
<td>0.254</td>
<td>0.015</td>
<td>0  2  0.01</td>
</tr>
<tr>
<td>Cholangiocarcinoma a</td>
<td>7</td>
<td>1.306</td>
<td>0.087</td>
<td>0.015</td>
<td>0  2  0.01</td>
</tr>
<tr>
<td>5. PVTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>1.422</td>
<td>0.194</td>
<td>0.003</td>
<td>0  3  6</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>0.869</td>
<td>0.077</td>
<td>0</td>
<td>4  13  5</td>
</tr>
<tr>
<td>6. Recurrence or metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>1.451</td>
<td>0.211</td>
<td>0.004</td>
<td>0  2  6</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>0.883</td>
<td>0.077</td>
<td>0</td>
<td>4  14  5</td>
</tr>
</tbody>
</table>

a Compared with HCC I and II.

b Including combined HCC.

Table 3
The factors influencing the postoperative HGF level

<table>
<thead>
<tr>
<th>Factor</th>
<th>POD 3</th>
<th>POD 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 cm</td>
<td>181 ± 18.9</td>
<td>121.9 ± 10.3</td>
</tr>
<tr>
<td>&gt;5 cm</td>
<td>137.2 ± 28</td>
<td>85.33 ± 10.2</td>
</tr>
<tr>
<td>Node cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>171 ± 20.2</td>
<td>113.4 ± 9.8</td>
</tr>
<tr>
<td>No</td>
<td>147.4 ± 28.1</td>
<td>94.1 ± 13.4</td>
</tr>
<tr>
<td>Surgical method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>114.3 ± 11.9</td>
<td>97.3 ± 9.6</td>
</tr>
<tr>
<td>Major</td>
<td>197.5 ± 23.6</td>
<td>113.2 ± 11.8</td>
</tr>
<tr>
<td>Recurrence or metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>124 ± 25.2</td>
<td>89.52 ± 15.96</td>
</tr>
<tr>
<td>No</td>
<td>176.1 ± 19.56</td>
<td>112.51 ± 9.04</td>
</tr>
</tbody>
</table>
expression (1.205 ± 0.152 ng/ml) versus 0.99 ± 0.127 ng/ml), normal limit (0.685 ± 0.115 ng/ml). Serum HGF concentrations above 0.91 ng/ml were detected in 17 patients (54.8%). The significantly high level of serum HGF was observed in groups with tumour more than 5 cm in diameter, PVTT, cholangiocarcinoma (including combined HCC) and poorly differentiated HCC. The results mentioned above suggest that some factors related to clinical-pathology of tumours affect pre- operative HGF level in serum, and that liver cancer cell may produce HGF. From the preoperative day to POD 10, the concentration of serum HGF had a decrease of percent (121.9 ± 10.2%) in the group with large tumours (>5 cm), but an elevation of percent (121.9 ± 10.3%) in the group with small tumours (<5 cm). As patients with large tumours had a higher level of HGF than those with small tumours before operation, following the resection of tumours, they should have a larger decrease of serum HGF than those with small tumour. However, liver regeneration post-resection may elevate HGF level, which reached a peak on POD 3, so the change of HGF between the groups of different tumour

### 4. Discussion

#### 4.1. The role of HGF and C-met in primary liver cancer

Over-expressions of HGF and c-met have been reported in liver cancerous tissues [9–11]. HGF is mainly secreted from mesenchymal tissues, while its receptor c-met has been demonstrated from various epithelial cells [12,13]. By binding to c-met, HGF causes constitutive activation of c- met and it’s downstream signalling pathways. In the liver, HGF mainly originates from hepatic satellite cells, sinusoidal endothelial cells and kupffer cells, etc., which is a paracrine fashion [10,14]. It is still in disagreement whether liver cancer cell expresses HGF or not [15,16]. Some studies have demonstrated that HGF could be secreted from hepatocyte, bile duct endothelial cell and tumour cell in the liver, which is an autocrine fashion [17–19]. Even if HGF expression can be found in the liver cancerous tissues, its positive rate was low [11]. Elevated HGF level usually occurs in serum [20]. So, we measured the HGF expression in serum but not in the tissue.

In this study, no significant correlation was observed between serum HGF and c-met expression in cancerous tissues; however, serum HGF level in the group with strong c-met expression was higher than the group with moderate expression (1.205 ± 0.152 ng/ml versus 0.99 ± 0.127 ng/ml), and the group with negative or weak c-met expression (1.205 ± 0.152 ng/ml versus 0.705 ± 0.147 ng/ml, p = 0.06), respectively, among which, there seem to be a tendency of correlation. Generally speaking, tumour cells express both HGF and c-met, constituting an autocrine loop, in which HGF level is usually correlated with c-met expression. The results in our study indicated that HGF probably functioned with c-met via paracrine loop. HGF can play a role in liver cancer, to some extent in a manner independent of its receptor c-met. Some other signal pathways can activate HGF. Recent reports have indicated that heparan sulphate proteoglycans such as syndecan-1 can be a receptor for HGF, which strongly promotes HGF-induced signalling through met, and this regulatory role of syndecan-1 may not be limited to the HGF/met pathway but may extend to other pathways driven by heparin-binding growth factors like epidermal growth factor and fibroblast growth factor 2 [21].

HGF, provided in a paracrine fashion, may serve not only as a tumour growth signal, but also as a potent inducer of invasive growth, metastasis and angiogenesis. Tumour cells can also produce some soluble factors that induce HGF expression [22,23]. Moreover, some extrahepatic cells such as lungs, kidney, spleen, etc. also produce HGF [24,25]. So, some patients with low expression of c-met may have a high level of serum HGF.

According to reports, c-met kinase activity can be regulated by other receptors through HGF-independent mechanisms, and it can be activated by adhesive receptors, such as various integrins and CD44, signal transducing receptors like Ron and the EGF receptor [26]. Interestingly enough, transgenic mice expressing c-met in hepatocytes develop HCC, despite the absence of detectable HGF expression [27]. These results indicate that over-expression of c-met may play a role in the genesis and maintenance of human HCC, provide an explanation for why HGF need not be present in tumours that over-express c-met [26].

In this report, serum HGF level was significantly higher in patients with liver cancer than in controls. According to the control value (0.685 ± 0.115 ng/ml), normal limit of serum HGF concentration was from 0.46 to 0.91 ng/ml (0.685 ± 1.96 × 0.115 ng/ml). Serum HGF concentrations above 0.91 ng/ml were detected in 17 patients (54.8%). The significantly high level of serum HGF was observed in groups with tumour more than 5 cm in diameter, PVTT, cholangiocarcinoma (including combined HCC) and poorly differentiated HCC. The results mentioned above suggest that some factors related to clinical-pathology of tumours affect pre-operative HGF level in serum, and that liver cancer cell may produce HGF.

#### Table 4

<table>
<thead>
<tr>
<th>C-met</th>
<th>n</th>
<th>Preoperative HGF (ng/ml)</th>
<th>S.E.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>0.705</td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0.99</td>
<td>0.127</td>
<td>0.209</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>1.205</td>
<td>0.152</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of HGF level among groups with different expression of c-met protein in primary liver cancer.

#### Table 5

<table>
<thead>
<tr>
<th>C-met protein</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Comparison of c-met protein in paracancerous tissues between the groups with and without cirrhosis.

Table 4

Comparison of HGF level among groups with different expression of c-met protein in cancerous tissues.
tumour recurrence [1,31]. We measured significantly higher proliferation after partial hepatectomy, thereby to support local ablation was observed in some patients. HGF has been shown to promote the growth of residual tumour after partial hepatectomy, and is involved in early tumour recurrence and metastasis.

In our study, over-expression of both HGF and c-met protein were detectable in most liver cancerous tissues (27/31, 87%), but only 16% (5/31) in the paracancerous tissues. The intensive expression of c-met mRNA was 100% (31/31) detectable in the cancerous tissues, but only 22.6% (7/31) in the paracancerous tissues. Both four cancerous tissues and two paracancerous tissues express only c-met mRNA, but no c-met protein. As the process of replication from mRNA to protein needs a series of steps, the tissue with mRNA expression may not be detected in its protein level.

In this study, the expression of c-met protein was correlated with PVTT, poorly differentiated HCC and cholangiocarcinoma (including combined HCC). Tumour size did not affect the c-met expression. Except for one patient with poorly differentiated tumour, all the other HCC patients with PVTT or poorly differentiated tumour had intensive or moderate expression of c-met. Especially, of the seven patients with cholangiocarcinoma, five had intensive c-met protein expression in cancerous tissues while the other two had moderate expression, and six patients had a preoperatively HGF level above 1ng/ml. These results showed that CC had over-expression of c-met and HGF. CC had a poor prognosis. HGF/c-met may have an important impact on its progression.

Intrahepatic recurrence and metastasis are the main factors affecting the surgical outcome for liver cancer [29,30]. Clinically, rapid growth of residual tumour after partial hepatectomy was observed in some patients. HGF has been suggested to initiate both hepatocyte and tumour cell proliferation after partial hepatectomy, thereby to support local tumour recurrence [1,31]. We measured significantly higher HGF levels in serum and c-met expression in cancerous tissues of the patients with tumour recurrence or metastasis than those without recurrence or metastasis. Of the eight patients with tumour recurrence or metastasis, preoperative concentration of serum HGF more than 1ng/ml was detected in seven, and strongly positive expression of c-met protein was observed in six, moderately positive in the other two. The results indicate that the patients with over-expressions of both HGF and c-met have a tendency of early tumour recurrence and metastasis post-resection.

In our study, over-expression of both HGF and c-met related to PVTT, cholangiocarcinoma and postoperative tumour recurrence (Table 2). Tumour size and differentiated grade of HCC had an effect on HGF level in serum, but no effect on c-met expression in cancerous tissues, which indicated that serum HGF may relate a lot to liver cancer progress. Because of uncommon c-met expression in normal liver tissues, high level of serum HGF after hepatectomy may play a main role in early tumour recurrence and metastasis.

4.2. HGF level and regeneration post-resection

Postoperatively, some factors influence serum HGF level. Liver is the major organ through which HGF is eliminated from circulation. As hepatectomy injures liver function, the clearance rate of HGF from circulation may decrease, which is one of the factors leading to elevated HGF level [32]. However, most importantly, the elevated HGF level may be the result of liver regeneration post-resection [5–8].

Ido et al. [33] reported that recombinant human HGF had only a half-life of 2.4 min. Due to the short half-life, serum HGF secreted from tumour should immediately decrease following the resection of tumour. Postoperatively, serum HGF is mainly affected by liver regeneration, which may reach a peak on POD 3. The proliferative process of liver lasts until it reaches a level where most of the original mass is recovered [7].

Moreover, our results showed that the surgical limit affected postoperative HGF level. Comparing POD 3 with preoperative day, the concentration of serum HGF had a higher elevation of percent in patients with major resection than in patients with local resection. Partial hepatectomy will induce liver injury, which is a signal leading to the generation of HGF. By the action of HGF, DNA synthesis in liver is promoted, which ultimately results in repair and regeneration of the injured liver. Therefore, HGF plays an important role in the repair and regeneration of liver after hepatectomy or injury [5–8]. Liver cirrhosis is another factor influencing the HGF and c-met expression. We found that patients with cirrhosis had a higher preoperative HGF level in serum and a higher rate of positive c-met-expression in paracancerous tissues than those without cirrhosis. As cirrhotic liver is accompanied with regeneration, it has usually expression of HGF and c-met [34,35].

In patients with partial hepatectomy, elevated HGF level resulting from hepatocyte proliferation may last for a period. In this report, HGF concentration on POD 10 was still significantly higher than controls. Persistent over-expression of HGF leads to its excessive reaction with c-met, providing malignant tumour cells with kinetic and aggressive traits, which may promote the growth of remnant tumour and hepatocarcinogenesis of normal liver [17,31]. It has been suggested that HGF plays a bi-functional role in the invasive behaviour of various tumours, and also in tissue repair and regeneration in reaction to tissue damage [36]. HGF is a potent stimulator of DNA synthesis in hepatocytes and interacts with other growth factors. The HGF/c-met system is involved in liver regeneration. Human liver regeneration is
influenced by the extent of resection, and also by the condi-
tion of the liver.
After partial hepatectomy, the increased tyrosine phospho-
rylation of c-met is found in the hepatocytes. HGF stimulates
the growth and migration of HCC cells through activation of
c-met and MAPKs, and it is involved in malignant behaviour
of cancer cells by enhancing invasion and metastasis [37,38].
The system of HGF/c-met has an important role in the
progress and metastasis of primary liver cancer. Inhibition of
HGF/c-met signals transduction system may be a key mea-
sure to prevent tumour growth and metastasis [39,40].

Conflict of interest statement
None declared.

References
[4] Taniguchi M, Takeuchi T, Nakamura T, Watanabe T, Sat0 K. Molec-
ular process in acute liver injury and regeneration induced by carbon
atic oval cells and possibly promotes the differentiation in a 2-
acetylaminoflavone/partial hepatectomy model in rats. J Gastroen-
[11] Liao YQ, Wu MC, Cong WM. Gene expression of hepatocyte growth fac-
[14] Giarriult J, Castroviejo M, Balbaid C, Desmouliere A, Rosenbaum J. Hepatocarcinoma cell stimulate hepatocyte growth factor secre-
[16] Bilencki B, Habelan AN, Demczew M. Hepatocyte growth factor in patients with three different stages of chronic liver disease includ-
[17] Horiuchi N, Takayama H, Teyoya M, Otsuka T, Fukuoka T, Mor-
gi G, et al. Hepatocyte growth factor promotes hepatocarcinogen-
esis through c-Met autocrine activation and enhanced angiogene-
[20] Dzianowska J, Zolitzch D, Polanisky L, Zajac L, Sitorzewski D, Lukom-
[26] Danilovskikh-Migalovk A, Zhar B. Dysregulation of Met recep-
[27] Wang R, Ferreri LD, Faouzi S, Maher J, Bishor JM. Activation of the Met receptor by cell attachment induces and sustains hepatocell-


