Detection of serum CD44v6, HMGB1, GP73 contents of liver cancer patients and its correlation with JAK–STAT pathway in tumor tissue

Ying-Xiang Yin
Department of Clinical Laboratory, the People’s Hospital of Leling City; Leling City, Shandong Province

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ABSTRACT

Objective: To study the serum CD44v6, HMGB1, GP73 contents of liver cancer patients and its correlation with JAK-STAT pathway in tumor tissue. Methods: 60 cases of patients diagnosed as primary liver cancer in our hospital from June 2013 to August 2014 were chosen as the observation group, 60 cases of healthy people judged by health examination in our hospital during the same period were chosen as the control group. Then serum was collected and CD44v6, HMGB1, GP73 contents were assayed, hepatocellular carcinoma tissue and paracancerous tissues were collected and JAK-STAT signal molecules and downstream regulatory gene were assayed. Results: (1) serum indexes: compared with serum hepatocellular carcinoma marker molecules, CD44v6, HMGB1, GP73 contents in serum of the observation group were lower; (2) JAK-STAT pathway: JAK1, JAK2, STAT1, STAT3, STAT5 expressions in hepatocellular carcinoma tissue were higher than those in paracancerous tissues; (3) signaling pathway regulates gene: TCF21, Pim1, VEGF, HIF-1, ICAM-1, VCAM-1 expression in hepatocellular carcinoma tissue that were higher than those in paracancerous tissues; (4) serum CD44v6, HMGB1 and GP73 contents were positively correlated with JAK1, JAK2, STAT1, STAT3, STAT5 expression. Conclusion: serum tumor marker molecules CD44v6, HMGB1 and GP73 contents of liver cancer patients abnormally increase and have correlation with the activation of JAK-STAT pathway in hepatocellular carcinoma tissue.

Primary liver cancer is a common malignant tumor of digestive system, which has a high degree of malignancy, poor prognosis and low survival rate. Serological indexes have been the ideal methods to judge the state of primary liver cancer, such as recently developed CD44v6, HMGB1 and GP73 serum marker, which is more and more used to evaluate the state of liver cancer. At present, the molecular mechanism of liver cells canceration have not been fully elucidated, thus to explore it is not only conducive to in-depth understanding of the disease, but also helps to fully explain the change of serum markers. JAK-STAT signal pathway is an important pathway in proliferation, apoptosis and angiogenesis process, which is associated with the occurrence of many malignant tumors. In the following study, the author analyzed the relationship between the serum contents of CD44v6, HMGB1 and GP73 in liver cancer patients and tumor tissue JAK-STAT pathway.

1. Materials and methods

1.1 Subjects

60 cases of primary liver cancer patients were selected during June 2013 to August 2014 in our hospital as the observation group, 60 cases of clearly healthy people over the same period as the control group. Patients with hepatocellular carcinoma were all pathologically diagnosed and have not received radiotherapy or chemotherapy. People in the control group were considered healthy and no one has chronic hepatitis and other diseases history. The two groups of people were informed subjects of the research and signed the informed consents. In the observation group: Male/female was 31/29, aged 57.95±7.42; the control group: Male/female was 35/25, aged 58.08±6.94. No statistically significant differences were found...
between the two groups of patients with general information \((P > 0.05)\).

1.2 Methods

1.2.1 Serum sample and liver tissue sample collection methods

5 mL peripheral blood of the observation group patients were collected before operation, while the same amount of blood were collected when the people took part in the control group. The blood were centrifuged to obtain serum, which then preserved at \(-80^\circ C\). During operation, the hepatocellular carcinoma tissues and adjacent normal tissues were collected into liquid nitrogen; the tissues were rapidly frozen and then saved at \(-80^\circ C\).

1.2.2 Testing indexes and methods

ELISA was used to determine the contents of CD44v6, HMGB1 and GP73 in serum. The mRNA contents of JAK1, JAK2, STAT1, STAT3, STAT5, TCF21, Pim1, VEGF, HIF-1 alpha, ICAM-1, VCAM-1 in liver tissues were determined by fluorescence quantitative PCR.

1.2.3 Statistical analysis method

SPSS18.0 software was used to input data. T test was performed on the measurement data. When \(P < 0.05\) the differences were decided as they have statistical significance.

2. Results

2.1 Serological indexes

Serological detection is an ideal method to determine the state of primary liver cancer patients. CD44v6, HMGB1 and GP73 are all ideal serum tumor markers. The author collected serum samples of primary liver cancer patients and healthy controls, and detected the contents of above mentioned serum tumor markers using ELISA. The analysis of t test showed that: compared to the control group, molecular markers in serum such as CD44v6, HMGB1 and GP73 contents was higher in the observation group. *: The differences were statistically significant \((P < 0.05)\).

![Figure 1: Comparison between the observation group and the control group of patients with serum tumor marker contents: after collection the serum tumor markers were detected by enzyme linked immunosorbent assay. The results showed that compared to the control group, CD44v6, HMGB1 and GP73 contents were higher in the observation group. *: The differences were statistically significant \((P < 0.05)\).](image)

2.2 Expressions of signal molecules in JAK–STAT signal pathway in tumor tissue

The abnormal activation of JAK-STAT signaling pathway is closely related to the carcinogenesis of hepatic cells, including JAK1, JAK1, JAK2, STAT1, STAT3, STAT5 and other signal molecules are involved in the occurrence of liver carcinoma. Therefore, the author collected the hepatocellular carcinoma tissues and adjacent normal tissues in the operation, to which mRNA were extracted and JAK-STAT signal molecules contents were detected by fluorescence quantitative PCR. T test analysis results showed that the expressions of JAK1, JAK2, STAT1, STAT3, STAT5 in hepatocellular carcinoma tissues were higher than those in the adjacent normal liver tissues. The differences had statistical significance \((P < 0.05)\).

2.3 Gene expressions in the regulation of JAK–STAT signaling pathway in tumor tissues

JAK-STAT signal pathway regulated genes including proliferation and apoptosis that regulate gene, angiogenesis gene and cell adhesion gene. Therefore, the author collected the hepatocellular carcinoma tissues and adjacent normal tissues in the operation, to which mRNA were extracted and gene expression in the regulation of JAK-STAT signaling pathway were detected by fluorescence quantitative PCR.
Table 2
Gene expressions in the regulation of JAK-STAT signaling pathway in tumor tissues

<table>
<thead>
<tr>
<th>TCF21</th>
<th>Pim1</th>
<th>VEGF</th>
<th>HIF-1</th>
<th>ICAM-1</th>
<th>VCAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver cancer tissues</td>
<td>245.62±38.25</td>
<td>193.46±28.56</td>
<td>276.52±36.71</td>
<td>291.38±41.03</td>
<td>316.72±44.39</td>
</tr>
<tr>
<td>para-cancerous tissues</td>
<td>100±17.03</td>
<td>100±18.51</td>
<td>100±16.89</td>
<td>100±17.79</td>
<td>100±15.92</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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T test and analysis results showed that TCF21, Pim1, VEGF, HIF-1 alpha, ICAM-1, VCAM-1 expressions in hepatocellular carcinoma tissues were higher than the adjacent normal liver tissues. The differences were statistically significant (P < 0.05).

3. Discussion

Serum liver cancer markers contents can determine the activity of liver cancer, which has the advantages of simple operation and good repeatability. CD44 is a trans-membrane glycoprotein widely expressed in a variety of cell surface, which has mediated cell adhesion and cell matrix adhesion effect. According to different exon gene structure, CD44 gene can be divided into standard type CD44s and mutant CD44v. The former is mainly expressed in normal cells, while the latter is mainly expressed in malignant cancer cells and is closely related with cancer cells’ malignant biological behavior[1].

Golgi protein 73 (GP73) in recent years is a serum marker used to determine the state of liver cancer. Under physiological conditions it is mainly located at the cis-face of the Golgi[2], when the liver cell starting malignant proliferation, GP73 will shift into cytoplasm and be released into the blood circulation, thus cause GP73 content in serum increase[3]. High mobility group protein B1 (HMGB1) is a newly discovered tumor marker, which has a high migration speed in polyacrylamide gel electrophoresis. The earliest studies regarded HMGB1 as a novel inflammatory mediator and related to inflammatory reaction and autoimmune reaction[4]. Recent studies showed that HMGB1 can promote angiogenesis factor secretion, recruit endothelial cells, activate p38 and JNK signaling pathways, and is closely related to the occurrence of liver cancer[5]. Through the analysis of serological index we found that serum markers CD44v6, HMGB1 and GP73 contents in the observation group were higher than in the control group. This suggests that abnormal increase of CD44v6, HMGB1 and GP73 contents in serum of liver cancer patients is closely related to liver cancer disease states.

In recent years, researches on primary liver cancer pathogenesis gradually went in depth. A variety of signaling pathways activation areas considered closely related with the carcinogenesis of liver cells. JAK-STAT signaling pathway is important to the regulation of cell proliferation, differentiation and angiogenesis, and is closely related to a variety of physiological and pathological processes in the body[6]. Janus kinase (JAK) is the kind of highly conserved non-receptor tyrosine kinases in the process of evolution, which includes JAK1, JAK2, JAK3 and TYK2, play receptor coupling and catalytic functions through the JH domain. Signal transducer and activator of transcription (STAT) is a downstream substrate of JAK family, containing DNA binding domain, tyrosine phosphorylation site domain, SH2 domain, C-terminal transactivation domain and so on. They are activated by JAK molecules and the form two dimers, then go into nucleus and directly combined with target gene in DNA regulatory regions, and the recruit of co-regulators or transcription factor can too[7]. In the process of liver cells canceration, under the regulation of extracellular signal molecules, such as interferon, interleukin and insulin-like growth factor, JAK-STAT signal pathway activated, related signaling molecules expressions abnormally increased and involved in the cell proliferation, invasion, cell cycle of cancer cells[8]. Expressions of JAK-STAT signal molecules in hepatocellular carcinoma tissues and para-carcinoma tissues were detected by fluorescence quantitative PCR in this study. The results are the expressions of JAK1, JAK2, STAT1, STAT3, STAT5 in hepatocellular carcinoma tissues that are higher than those in para-carcinoma tissues. This suggests that the abnormal activation of JAK-STAT signaling pathway is closely related to liver cells canceration.

Current research suggests that JAK-STAT signaling pathway regulated genes including proliferation and apoptosis regulation gene, angiogenesis gene and cell adhesion gene. The proliferation and apoptosis out of control are important mechanisms to cause the malignant proliferation of liver cells. Transcription factor 21 (TCF21) is a kind of newly discovered apoptosis gene, impacting on the promoter of KISS gene, promoting the gene expression and enhancing the apoptosis effects[9]. Pim-1 is a proliferation promoting gene belonging to the proto-oncogene, which can encode serine/threonine protein kinase and block the cells into apoptosis pathways by inhibiting the tumor suppressor gene P53, thus it has a promote proliferation role[10]. Vascular endothelial growth factor (VEGF) and hypoxia inducible factor (HIF-1 ) are important molecules involved in angiogenesis[11]. VEGF is known as the most strong pro-angiogenic molecules, which can induce tumor local vascular bed form and provide adequate blood supply to cancer cells proliferation process[12]. HIF-1 is a nuclear transcription factor regulated by JAK-STAT, which turned into nuclear can regulates the expression of related genes[13]. Inter cellular adhesion molecule-1...
(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are important members of the family of adhesion molecules. The former can mediate adhesion between cancer cells and normal tissue cells, while the later can mediate adhesion of cancer cells and vascular endothelial cells. Over expressions of the two are related to liver cancer hematogenous metastases and local invasion[14-15]. This study detected the regulate genes expression amount of JAK-STAT pathway by fluorescence quantitative PCR. The results showed that the expressions of TCF21, Pim1, VEGF, HIF-1 alpha, ICAM-1, VCAM-1 in hepatocellular carcinoma tissues are higher than those in the adjacent normal liver tissues.

After being comprehensively discussed above, we can draw the conclusion that there are abnormal increases of the contents of serum tumor markers CD44v6, HMGB1, GP73 in liver cancer cells and is closely related to the activation of JAK-STAT pathway in cancer tissues.

References