Detection of serum tumor marker contents of liver cancer patients and its correlation with JAK–STAT pathway in tumor tissue

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Objective: To study the serum tumor marker contents of liver cancer patients and its correlation with HAK-STAT pathway in tumor tissue. Methods: 50 cases of primary liver cancer patients diagnosed in our hospital from August 2013 to November 2014 were enrolled in liver cancer group; 50 cases of healthy persons received physical examination in our hospital during the same period were enrolled in healthy group. Then serum was collected and Livin, Xiap, Pim-1, ICAM-1, VCAM-1, CD44v6, DNMT1, DNMT2, DNMT3A, DNMT3B, HDAC1 and HDAC5 were detected; liver tissue was collected and JAK1, JAK2, STAT1, STAT3 and STAT5 were detected. Results: (1) Proliferation and adhesion molecules: compared with serum proliferation and adhesion molecule contents of the healthy group, serum Livin, Xiap, Pim-1, ICAM-1, VCAM-1 and CD44v6 contents of liver cancer group were higher; (2) Methyltransferase and histone deacetylase: compared with serum methyltransferase and histone deacetylase contents of healthy group, serum mRNA contents of DNMT1, DNMT2, DNMT3A, DNMT3B, HDAC1 and HDAC5 of liver cancer group were higher; (3) Signal molecules: compared with JAK-STAT signal molecule contents in adjacent normal liver tissue, mRNA contents of JAK1, JAK2, STAT1, STAT3 and STAT5 in hepatocellular carcinoma tissue were higher. Conclusion: Contents of serum proliferation and adhesion molecules, methyltransferase and histone deacetylase abnormally increase, which is closely related to the abnormal activation of JAK-STAT pathway in tumor tissue.

Molecular mechanism of primary liver cancer is quite complex. Enhanced cancer cell proliferation and adhesion as well as abnormal regulatory process of intranuclear DNA methylation and histone deacetylation are very important features, with specific expressions of increased contents of serum proliferation and adhesion molecules as well as abnormal expressions of methyltransferase and histone deacetylase. In clinical practice, these molecules are used as serum markers to judge malignant degree of hepatocellular carcinoma. However, pathogenesis of hepatocellular carcinoma as well as abnormal expressions of above molecules has not been clarified yet. Abnormal activation of JAK-STAT signal pathway is a possible cause. In the following research, serum tumor marker contents of liver cancer patients and its correlation with HAK-STAT pathway in tumor tissue were analyzed.

I. Materials and methods

I.1 Materials

50 cases of primary liver cancer patients diagnosed in our hospital from August 2013 to November 2014 were enrolled in liver cancer group. All patients were diagnosed by clinical symptoms, signs and pathologic biopsy and had never received radiation and chemotherapy. 50 cases of healthy persons received physical examination in our hospital during the same period were enrolled in healthy group. Enrolled persons were healthy and didn’t have chronic hepatitis and other diseases. Both groups were notified.
of research related matters and signed informed consents. Liver cancer group included 32 males and 18 females with age range of 61.49±7.84; healthy group included 30 males and 20 females with age range of 60.85±7.38. There were no statistical differences between two groups’ general data (P>0.05).

1.2 Methods

1.2.1 Serum collecting methods
After admission, 5ml of peripheral venous blood of liver cancer patients was collected; 5ml of peripheral venous blood of healthy persons was collected during physical examination; blood was centrifuged. Upper serum was collected, put in centrifuge tube and preserved at -80°C.

1.2.2 Liver tissue collecting methods
Liver tissue of liver cancer patients was collected during surgery, including liver cancer tissue and adjacent normal liver tissue. Different tissues were confirmed by pathological examination, frozen by liquid nitrogen and preserved at -80°C.

1.2.3 Detecting indexes and methods
Trizol was used to extract mRNA from blood specimen and liver tissue. Then fluorescence quantitative PCR was used to amplify Livin, Xiap, Pim-1, ICAM-1, VCAM-1, CD44v6, DNMT1, DNMT2, DNMT3A, DNMT3B, HDAC1, HDAC5, JAK1, JAK2, STAT1, STAT3 and STAT5. Take-off cycle number Ct values were read to calculate mRNA contents.

1.2.4 Statistical methods
Data detected through PCR were input by SPSS18.0 software, comparison of pair wise differences for t test. Differences were considered to be statistically significant at a level of P<0.05.

2. Results

2.1 Serum proliferation and adhesion molecule contents
Proliferation and adhesion are important malignant biological behaviors of primary liver cancer. Proliferation process is regulated by pro-proliferation genes and adhesion process is regulated by adhesion molecules. In the research, PCR was used to amplify proliferation genes and adhesion molecules. Then t test was carried out and results showed that compared with serum proliferation and adhesion molecule contents of healthy group, serum Livin, Xiap, Pim-1, ICAM-1, VCAM-1 and CD44v6 contents of liver cancer group were higher. Figure.1 is for details.

![Figure 1: mRNA contents of proliferation and adhesion molecules in serum specimen of liver cancer group and healthy group. Serum Livin, Xiap, Pim-1, ICAM-1, VCAM-1 and CD44v6 contents of liver cancer group were higher than those of healthy group. *: there were differences between liver cancer group and healthy group.](image)

2.2 Serum methyltransferase and histone deacetylase contents
Potential causes of abnormal expressions of proto-oncogene and tumor suppressor gene may be abnormal DNA methylation and deacetylation degrees; methyltransferase and histone deacetylase are key enzymes that regulate DNA methylation and deacetylation processes and are closely related to the occurrence of liver cancer. In the research, PCR was used to detect methyltransferase and histone deacetylase contents. Then t test was carried out and results showed that compared with serum methyltransferase and histone deacetylase contents of healthy group, serum mRNA contents of DNMT1, DNMT2, DNMT3A, DNMT3B, HDAC1 and HDAC5 of liver cancer group were higher. Differences had statistical significance (P<0.05).

2.3 JAK–STAT signal molecule contents in liver cancer tissue
The occurrence of liver cancer is related to abnormal expressions of JAK-STAT signal pathway related molecules. JAK1, JAK2, STAT1, STAT3 and STAT5 are signal molecules that are closely related to liver cell cancreration. In the research, PCR was used to

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<th>Table 1: Comparison of serum methyltransferase and histone deacetylase contents</th>
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amplify JAK-STAT signal molecules. Then the test was carried out and results showed that compared with JAK-STAT signal molecule contents in adjacent normal liver tissue, mRNA contents of signal molecules JAK1, JAK2, STAT1, STAT3 and STAT5 in hepatocellular carcinoma tissue were higher. Figure 2 is for details. Differences had statistical significance ($P<0.05$).

### 3. Discussions

Proliferation and adhesion are important malignant biological behaviors of primary liver cancer and are pathological and physiological basis that cause local recurrence and distant metastasis of liver cancer. Proliferation process is regulated by pro-proliferation genes and adhesion process is regulated by adhesion molecules. Therefore, pro-proliferation molecules and adhesion molecules are used as tumor markers to judge liver cancer condition in clinical setting. Both Livin and Xiap belong to apoptosis-inhibiting genes with pro-proliferation effect; proteins expressed by these two genes can directly inhibit apoptosis-executing molecules Caspase3 with pro-proliferation effect; proteins expressed by these two are important molecules that cause distant metastasis of cancer cells; vascular cell adhesion molecule-1 (VCAM-1) can mediate adhesion of liver cancer cells with vascular endothelial cells. These two are important molecules that cause distant metastasis of cancer cells. CD44 is a transmembrane glycoprotein. CD44s and CD44v belong to standard type and variant type respectively. Difference between the two molecules is gene exon structure; CD44v is mainly expressed in malignant cells and can mediate adhesion of cancer cells to adjacent tissue. The research analyzed serum proliferation molecule and adhesion molecule contents and found that serum Livin, Xiap, Pim-1, ICAM-1, VCAM-1 and CD44v6 contents of liver cancer group were higher than those of healthy group, which indicated that pro-proliferation molecule and adhesion molecule contents of liver cancer patients abnormally increased.

Epigenetics is a new study hotspot of gene expression and regulation in recent years. Both DNA methylation and histone deacetylation can cause changes of gene stability and transcription regulation. In case of proto-oncogene methylation, proto-oncogene will be activated; tumor suppressor gene deacetylation will cause tumor suppressor gene inactivation, based on which, cell canceration is caused. DNA methyltransferase (DNMT) directly participates in DNA methylation process. DNMTs can use S-adenyllyl-L-methionine as donor and transfer the methyl to the fifth carbon atom of DNA cytosine nucleotides, cytosine becoming 5-methylcytosine. When methylation process occurs in promoter region of tumor suppressor gene, transcription factors cannot properly identify the promoter regions, tumor suppressor gene expressions are blocked and cell canceration risk increases. DNMT1, DNMT2, DNMT3a and DNMT3b are four main DNMTs and can regulate methylation degree of gene promoter region. Histone deacetylases (HDAC) can regulate histone deacetylation degree in cells. HDAC1 and HDAC5 can cause histone deacetylation, and then tightly bind with tumor suppressor gene DNA, preventing expressions of tumor suppressor genes. The research analyzed serum methyltransferase and histone deacetylase contents to reflect DNA methylation degree and histone deacetylation degree. Results showed that serum mRNA contents of DNMT1, DNMT2, DNMT3A, DNMT3B, HDAC1 and HDAC5 of liver cancer group were higher than those of healthy group, which indicated that there were abnormal methylation degree and deacetylation degree in liver cancer patients.

In liver cell canceration process, expressions of proliferation and adhesion molecules, methyltransferase and histone deacetylase are regulated by multiple signal pathways. Abnormal signal pathway function will cause abnormal expressions of related analysis. JAK-STAT signal pathway includes two types of molecules, Janus kinase (JAK) as well as signal transducers and activator of transcription (STAT). It participates in the regulation of multiple cell biological behaviors. In JAK family, JAK1 and JAK2 are related to liver cell canceration and regulate downstream molecule STAT through JH domain; STAT family include DNA-binding domain, tyrosine phosphorylation domain, SH2 domain and carboxyl-terminal transcription function domain. The active forms are dimmers. When trans-located and entering nucleus, they can recruit multiple transcription factors and co-regulators, implementing the regulation to target gene expressions. When liver cells are influenced by interleukins, interferons and other extracellular signal molecules, intracellular JAK-STAT signal pathway will be activated. Excessive expressions of related signal molecules will
cause abnormal expressions of proliferation and adhesion molecules, methyltransferase and histone deacetylase. The research used fluorescence quantitative PCR to detect JAK-STAT signal molecule contents in liver tissues and results showed that mRNA contents of JAK1, JAK2, STAT1, STAT3 and STAT5 in hepatocellular carcinoma tissue were higher than those in adjacent normal liver tissue and were positively correlated with serum Livin, Xiap, Pim-1, ICAM-1, VCAM-1, CD44v6, DNMT1, DNMT2, DNMT3A, DNMT3B, HDAC1 and HDAC5.

In conclusion, contents of serum proliferation and adhesion molecules, methyltransferase and histone deacetylase abnormally increase, which is closely related to the abnormal activation of JAK-STAT pathway in tumor tissue.

References


