The analysis of γ-glutamyl transpeptidase gene in different type liver tissues

Guo-Qing Han, Cheng-Yong Qin, Rong-Hua Shu

AIM: To probe the value of γ-glutamyl transpeptidase (GGT) messenger RNA in monitoring canceration of liver cells and for early diagnosis of hepatocellular carcinoma (HCC), by researching the types of GGT messenger RNA (GGTmRNA) in liver tissues and peripheral blood of different hepatopathy.

METHODS: The three types of GGTmRNA (A, B, C) in liver tissues and peripheral blood from the patients with HCC, noncancerous hepatopathy, hepatic benign tumor, secondary carcinoma of liver, and healthy persons were detected by reverse-transcription polymerase chain reaction (RT-PCR).

RESULTS: (1) In normal liver tissues, type A was predominantly found (100.00 %), type B was not found, type C was found occasionally (25.00 %). (2) The distribution of types of GGTmRNA in liver tissues with acute hepatitis, chronic hepatitis, cirrhosis, alcoholic hepatic hepatitis was similar as in normal liver tissues (P>0.05), but type B was found in 3 of 18 patients with chronic hepatitis (16.67 %), and also in 3 of 11 patients with cirrhosis (27.27 %). (3) There was no significant difference of types of GGTmRNA between liver tissues with hepatic benign tumor, secondary carcinoma of liver and normal liver tissues (P>0.05). (4) Type B was predominant in cancerous tissues with HCC (87.5 %), the prevalence of type B in cancerous tissues was significantly higher than that in normal liver tissues (0/12) (P<0.05), but the prevalence of type A in cancerous tissues (46.88 %) was significantly lower than that in normal liver tissues (100.00 %) (P<0.05), and the prevalence of type C (6.25 %) in cancerous was the same as that in normal liver tissues (25.00 %) (P>0.05). In noncancerous tissues of livers with HCC, the main types were type A and type B, the prevalence of type A (85.71 %, 90.48 %) and type C (14.29 %, 9.52 %) in noncancerous tissues of livers with HCC was similar as that in normal liver tissues (A: 100.00 %; C: 25.00 %) (P>0.05), but the prevalence of type B (80.95 %, 76.19 %) in noncancerous tissues of livers with HCC was significantly higher than that in normal liver tissues (0/12) (P<0.05). (5) The prevalence of type B (37.5 %) in peripheral blood with HCC was higher than that in normal person (0/12) (P<0.05). In peripheral blood, type B was found in 4 of 11 cases of HCC with serum AFP negative.

CONCLUSION: The shift of types of GGTmRNA from A to B in liver tissues may be closely related to the development of HCC, and the analysis of GGT gene may provide a useful tool for early diagnosis of HCC.
paracancerous tissues (to the brim of carcinoma 3-5 cm) and
distal cancerous tissues (to the brim of carcinoma >5 cm) were
respectively obtained during surgery from 21 patients with
HCC, and the cancerous tissues and noncancerous tissues were
obtained by needle liver biopsy with help of ultrasound B from
the other patients of HCC (11 cases). Liver tissues were
obtained by needle liver biopsy in the other groups, non-tumor
tissues were merely obtained in hepatic benign tumor and
secondary carcinoma of liver. All the tissue specimens were
immediately refrigerated by liquid nitrogen for 1 hour, and
then were transferred to refrigerator at -80 °C for future use.

Blood samples: peripheral blood was obtained from each
person at morning before breakfast, anticoagulated by sodium
citrate solution, and then peripheral blood mononuclear cells
(PBMC) were separated from blood, total RNA was extracted
from PBMC, cDNA was synthesized with reverse transcription,
and then reserved in refrigerator at -80 °C.

**Total RNA extraction**

Total RNA was extracted by using TRIzol (from TECH-LINE
company) and the purity of RNA was tested with ultraviolet
spectrophotometer of DAOJUN UR-2201.

**Detection of the types of GGTmRNA by RT-PCR**

cDNA was synthesized with RNA, reverse transcriptase (M-
MLV) and random primer (Oligdts). then cDNAs of
GGTmRNA were amplified by PCR using polymerase and
three different primer sets which were specific for the three
GGTmRNA types. Nucleotide sequences of the primer sets to
each type of GGTmRNAs are: type A sense 5’- CAC AGG
GGTmRNA was similar as in normal livers (.81.82 %) was similar as in normal livers (>0.05); however
the prevalence of type B (80.95 % , 76.19 % , 72.73 %) was significantly higher than in normal liver tissues (>0.05); the prevalence of type C was similar as in normal livers (>0.05).

The relation between types of GGTmRNA and size of HCC:
The prevalence of type A in cancerous tissues of larger sized HCC
is lower than in that of smaller sized HCC, the monocogenic pattern
of type B tended to be found more frequently in larger sized HCC,
but the difference was not significant (>0.05 ). Table 2.

**Table 1 Incidence of Different GGTmRNA types in Livers of Each Group**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Samples</th>
<th>Type of GGTmRNA</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal</td>
<td>12</td>
<td></td>
<td>12 (100%)</td>
<td>0</td>
<td>3 (25.00%)</td>
</tr>
<tr>
<td>2. Acute hepatitis</td>
<td>15</td>
<td></td>
<td>14 (93.33%)</td>
<td>1 (6.67%)</td>
<td>2 (13.33%)</td>
</tr>
<tr>
<td>3. Chronic hepatitis</td>
<td>18</td>
<td></td>
<td>16 (88.89%)</td>
<td>3 (16.67%)</td>
<td>4 (22.22%)</td>
</tr>
<tr>
<td>4. Cirrhosis</td>
<td>11</td>
<td></td>
<td>9 (81.82%)</td>
<td>3 (27.27%)</td>
<td>2 (18.18%)</td>
</tr>
<tr>
<td>5. Alcoholic hepatopathy</td>
<td>13</td>
<td></td>
<td>12 (92.31%)</td>
<td>1 (7.66%)</td>
<td>3 (23.08%)</td>
</tr>
<tr>
<td>6. Hepatic benign tumor</td>
<td>10</td>
<td></td>
<td>10 (100%)</td>
<td>0</td>
<td>1 (10.00%)</td>
</tr>
<tr>
<td>7. Secondary carcinoma of liver</td>
<td>13</td>
<td></td>
<td>11 (84.62%)</td>
<td>0</td>
<td>3 (23.08%)</td>
</tr>
<tr>
<td>8. HCC</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancerous tissues</td>
<td>32</td>
<td></td>
<td>15 (46.88%)</td>
<td>28 (87.5%)</td>
<td>2 (6.25%)</td>
</tr>
<tr>
<td>Adjacent Paracancerous tissues</td>
<td>21</td>
<td></td>
<td>18 (85.71%)</td>
<td>17 (80.95%)</td>
<td>3 (14.29%)</td>
</tr>
<tr>
<td>Distal Cancerous tissues</td>
<td>21</td>
<td></td>
<td>19 (90.48%)</td>
<td>16 (76.19%)</td>
<td>2 (9.52%)</td>
</tr>
<tr>
<td>Noncancerous tissues</td>
<td>11</td>
<td></td>
<td>9 (81.82%)</td>
<td>8 (72.73%)</td>
<td>1 (9.09%)</td>
</tr>
</tbody>
</table>

**Table 2 GGTmRNA and the size of HCC**

<table>
<thead>
<tr>
<th>Size of HCC</th>
<th>Number of cases</th>
<th>GGTmRNA type A</th>
<th>GGTmRNA type B</th>
<th>GGTmRNA type C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small sized HCC</td>
<td>8</td>
<td>5 (62.50%)</td>
<td>6 (75.00%)</td>
<td>5 (62.50%)</td>
</tr>
<tr>
<td>Large sized HCC</td>
<td>16</td>
<td>7 (43.75%)</td>
<td>15 (93.75%)</td>
<td>14 (87.50%)</td>
</tr>
<tr>
<td>Enormous sized HCC</td>
<td>8</td>
<td>3 (37.50%)</td>
<td>7 (87.50%)</td>
<td>7 (87.50%)</td>
</tr>
</tbody>
</table>

**GGTmRNA in peripheral blood**

In peripheral blood of 32 patients with HCC, type A was found
in 2 cases (6.25 %), type B was found in 12 cases, the
prevalence of type B (37.5 %) was significantly higher than normal (>0.05), type C was found in 1 case (3.13 %). In
peripheral blood of patients with acute hepatitis, type A was
found in 2 cases. In chronic hepatitis group, type A was found
in 1 case. Type B and C were not found in acute and chronic
hepatitis group. In the other groups, GGTmRNA was not found.
In 8 cases of small sized HCC, type B was found in 5
noncancerous tissues (62.5 %) and in peripheral blood of 2
cases (25 %). In the 8 cases, there were 3 patients with serum...
In 21 cases of HCC with AFP positive, type B was found in peripheral blood of 8 cases (38.1 %). In 11 cases of HCC with AFP negative, type B was found in peripheral blood of 4 cases (36.36 %). Type B was not significantly different between them (P>0.05). Therefore the prevalence of type B in peripheral blood was not related to the prevalence of AFP.

**DISCUSSION**

Hepatocellular carcinoma is one of the common malignant tumor[17-39], because of its severe malignance, quick development, early intrahepatic metastasis, mostly being combined with cirrhosis, frequent recurrence, the prognosis of HCC still remains dismal[30-41]. By now, surgery is still the most efficient treatment for HCC, but about 70 percent of patients with HCC lost the opportunity of surgery, since they did not go to see a doctor until the tumor reached an advanced stage, and HCC reoccurred more frequently after surgery, on the other hand, HCC is not susceptible to radiotherapy, chemotherapy and other synthetic treatments[42-44], so it is imperative to clarify the pathogenic mechanism of HCC and to find efficient methods for early diagnosis of HCC. The epidemiological studies suggested that the prevalence of HCC in patients with hepatopathy had been obviously increasing in China. At present, the pathogenic mechanisms of HCC are not well known, it is reported that the occurrence and development HCC may be a process of polygenic and multiple steps, which related to polygenic expression, such as repair of DNA, signal transduction, cell cycle regulation etc[45-52].

It is still difficult to monitor the canceration of liver cells in preneoplastic stage and early stage of HCC[53]. If we could monitor the changes of the structure and function of some genes, we would find the patients with high risk of HCC, forecast the possibility of occurrence of HCC before cytological changes, and then we could prevent, make a diagnosis and give treatment on molecular level.

It has been reported that HCC synthesizes and secretes many proteins, polypeptides or isoenzymes such as AFP, GGT etc. they may be used as important marks for the diagnosis of HCC. GGT is closely relate to biotransformation, metabolism of nucleic acid, and the occurrence of carcinoma, it may be used as a mark for detection of bibulosity and the canceration of liver cells. In humans, the activity of GGT is at a very high level in embryo period, after birth it declines to a very low level quickly, HCC expresses a large amount of GGT and unique GGT isoenzyme. However, its mechanisms are not sure whether or not some liver cells in liver tissues of these 6 cases had developed to the preneoplastic stage or changed to cancerous cells, and whether or not these 6 patients would developed to HCC in the future. It need further follow-up study to answer these questions.

Among the patients of hepatic benign tumor and secondary carcinoma, the distribution of GGT mRNA in livers tissues but not tumor tissues was similar as that in normal livers. The results suggested that the shift from type A to type B did not exist in liver tissues with benign tumor and secondary carcinoma.

The serum level of GGT is mostly higher in patients with alcoholic hepatopathy, but the distribution of types of GGT mRNA in these liver tissues was similar as in normal liver tissues. This result suggested that alcohol did not induce the shift of GGT mRNA.

Studies about small sized HCC have been the important incident in the history of HCC in the past 20 years. Early diagnosis and treatment are the keys to increase survival rate and decrease recurrence rate. The detection of serum alphafetoprotein (AFP) is an important method for early diagnosis of HCC, especially in patients with high risk of HCC[55,56]. However, the negative rate of AFP is higher in patients with small sized HCC. In present study, among the 8 patients with small sized HCC, type B was detected in noncancerous liver tissues of 5 patients, and in peripheral blood of 2 cases, however, AFP was positive in serum of only 2 patients. Moreover, in peripheral blood, type B of GGT mRNA was found in 4 of 11 HCC patients with AFP negative (36.36 %). These results suggested that the detection of unique type B of GGT mRNA may provide a useful tool for the diagnosis of the small sized HCC and HCC with AFP negative.

Since there are lots of RNA enzymes in blood plasma, RNA will be degraded by RNA enzymes as soon as it appears in plasma, so there are not dissociative GGT mRNAs in blood plasma in normal blood plasma. In this study, GGT mRNAs were not found in normal peripheral blood, however, among 32 cases of HCC, type B of GGT mRNA was found in peripheral blood of 12 cases (37.5 %), GGT mRNAs were not found in peripheral blood in other groups. So it may be inferred that cancerous cells exist in peripheral blood of this 12 patients. These results suggest that the shift of type B of GGT mRNA are closely related to the development of HCC, and that analysis of GGT mRNA expression may provide a useful tool for early diagnosis of HCC.

**REFERENCES**

1. Ma XD, Sui YF, Wang WL. Expression of gap junction genes connexin32 and connexin43 and their proteins in hepatocellular...
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Fu JM, Yu XF, Shao YF. Telomerase and primary liver cancer. Shijie Hua Huan Xioa Zzhi 2000; 8: 461-463.

19.  

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21.  
Tang YW, Yao XJ. Regulating effect of HCC cells on the activation of stellate cells. Shijie Hua Huan Xioa Zzhi 2001; 9: 202-204.

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Liang Y, Lu B, Cui ZF, Li XD, Guo YJ, Lu YJ. The expression of Fas/FasL in hepatocellular carcinomas. Shijie Hua Huan Xioa Zzhi 2001; 9: 1364-1369.

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56 Tian FZ. Tumor markers of hepatocellular carcinoma. Shijie Huaren Xiaohua Zazhi 2000; 8: 440-441

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