PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 February 28; 12(8):1175-1181
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Serum tumor markers for detection of hepatocellular carcinoma

Lin Zhou, Jia Liu, Feng Luo

Lin Zhou, Feng Luo, Division of Biotherapy for Cancer, Cancer Center, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China
Jia Liu, Cancer Center, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China
Co-correspondent: Lin Zhou
Correspondence to: Dr. Feng Luo, Division of Biotherapy for Cancer, Cancer Center, West China Hospital of Sichuan University, 37 Guoxue Xiang, Chengdu 610041, Sichuan Province, China. luofeng@medmail.com.cn
Telephone: +86-28-85423581
Received: 2005-08-31 Accepted: 2005-10-10

Abstract

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors and is the second most common cause of cancer death in China. Therefore, it is very important to detect this disease and the recurrence at its earlier period. Serum tumor markers, as the effective method for detecting hepatocellular carcinoma for a long time, could be divided into 4 categories: oncofetal antigens and glycoprotein antigens; enzymes and isoenzymes; genes; and cytokines. Serum alpha fetoprotein (AFP) is the most widely used tumor marker in detecting patients with hepatocellular carcinoma, and has been proven to have capability of prefiguring the prognosis. However, it has been indicated that AFP-L3 and DCP excel AFP in differentiating hepatocellular carcinoma from nonmalignant hepatopathy and detecting small hepatocellular carcinoma. Some tumor markers, such as human cervical cancer oncogene and human telomerase reverse transcriptase mRNA, have also been indicated to have higher accuracies than AFP. Furthermore, some other tumor markers, such as glypican-3, gamma-glutamyl transferase II, alpha-l-fucosidase, transforming growth factor-beta1, tumor-specific growth factor, have been indicated to be available supplementaries to AFP in the detection. AFP mRNA has been shown to correlate with the metastasis and recurrence of HCC, and it may be the most useful marker to prefigure the prognosis. Some other markers, such as gamma-glutamyl transferase mRNA, vascular endothelial growth factor, and interleukin-8, could also be used as available prognostic indicators, and the simultaneous determination of AFP and these markers may detect the recurrence of HCC at its earlier period.

Key words: Hepatocellular carcinoma; Serum tumor markers; Sensitivity; Specificity; Prognosis

Zhou L, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. *World J Gastroenterol* 2006; 12(8): 1175-1181

http://www.wjgnet.com/1007-9327/12/1175.asp

INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most frequent malignant tumors, is the second common cause of cancer death in China, where its mortality rate is 20.37/100 thousand[1]. Surgical resection is the most effective method for curing this disease, but a large amount of cases are not adapted to surgery because of their intrahepatic or distant metastases at the time of diagnosis. Furthermore, the long-term survival of postoperative HCC patients is unsatisfactory for the high incidence of recurrence. Therefore, it is very important to detect HCC and the recurrence at its earlier period. By reasons of convenience, inexpensiveness, and the satisfactory accuracy, serum tumor markers have been used as an effective method for detecting malignant tumors for a long time, and they could be valuable supplementaries to ultrasonography and computer tomography in the diagnosis of HCC. Using the appropriate single or combination of tumor markers may improve the effectiveness in screening HCC patients.

ONCOFETAL ANTIGENS AND GLYCOPROTEIN ANTIGENS

Alpha-fetoprotein and alpha-fetoprotein-L3

Alpha fetoprotein (AFP) is a fetal specific glycoprotein produced primarily by the fetal liver. Normally, its serum concentration falls rapidly after birth and its synthesis in adult life is repressed. However, greater than 70% of HCC patients have a high serum concentration of AFP because of the tumor excretion. Forty years after its discovery, serum AFP remains a most useful tumor marker in screening HCC patients. The serum concentration of 20 ng/mL is the most commonly used cut-off value to differentiate HCC patients from healthy adults in clinical researches. However, some investigations have showed that the cut-off value is fluctuant in different ethnic groups.

The best cut-off value of AFP has been reported to be 30 ng/mL (sensitivity of 65%, specificity of 89%) in Sicilian population compared with 200 ng/mL (sensitivity of 70%, specificity of 100%) in Burman population[2,3]. One of possible reasons for this difference is the diverse living circumstance which has a great influence on epidemiology. Besides the purpose of screening HCC, serum and tissues AFP could also be used as prognostic indicators[4]. HCC patients with a high AFP concentration ($\geqslant$ 400 ng/mL) tend to have greater tumor size, bilobar involvement, massive or diffuse types, portal vein thrombosis, and a lower median survival rate[5,6]. This is partially caused by the expression of ephrin-A1 (an angiogenic factor) and the ability of AFP to elicit the escape of carcinoma cells from the host's lymphocytes immune surveillance[7,8]. Though the measurement of AFP serves as an important tool in screening HCC patients, some reports have indicated that it has limited utility of differentiating HCC from benign hepatic disorders for its high false-positive and false-negative rates, and patients with acute exacerbation of viral hepatitis but no HCC may also have markedly increased AFP levels[9]. Using the cut-off value of 20 ng/mL to differentiate HCC from HCV-infected patients, sensitivities merely range from 41% to 65% with specificities of 80% to 94% correspondingly[10]. Moreover, the positive predictive value (PPV) of AFP is significantly lower in detecting HCC patients with viral etiology than that in detecting HCC patients with non-viral etiology (70% *vs* 94%, P <0.05), and it will not reach 100% in HCC patients with viral etiology unless their serum concentration of AFP is greater than 400 ng/mL[2,11]. Therefore, AFP is more useful in detecting HCC patients with non-viral etiology.

Total AFP can be divided into three different glycoforms, namely AFP-L1, AFP-L2 and AFP-L3, according to their binding capability to lectin lens culinaris agglutin (LCA). AFP-L1, as the non-LCA-bound fraction, is the major glycoform of AFP in the serum of nonmalignant hepatopathy patients. On the contrary, AFP-L3, as the LCA-bound fraction, is the major glycoform of AFP in the serum of HCC patients, and it can be detected in approximately 35% of patients with small HCC (< 3 cm), especially when the tumor mass is supplied by the hepatic artery. At the cut-off level of 15%, sensitivities of AFP-L3 in detecting HCC range from 75% to 96.9% with specificities of 90% to 92.0% correspondingly[3,12]. Furthermore, some clinical researches have indicated that the high percentage of AFP-L3 is closely related to poor differentiation and biologically malignant characteristics (especially portal vein invasion) of HCC[12,13], and HCC patients with positive AFP-L3 would have worse liver function, poorer tumor histology, and larger tumor mass[14]. Compared with those with serum concentration of des-gamma-carboxyprothrombin (DCP) over 100 mAU/mL, HCC patients with percentage of serum AFP-L3 over 15% also showed a higher incidence of infiltrative-type HCC with an irregular margin (P<0.05) and a higher frequency of poorly differentiated HCC (P<0.01)[15]. Therefore, it could be used as a valuable indicator of poor prognosis.

Glypican-3

Glypican-3 (GPC3) is a heparan sulfate proteoglycan anchored to the plasma membrane. It has been demonstrated to interact with growth factors and modulate their activities. The expression of GPC3 (at both mRNA and protein levels) in the serum of HCC patients is significantly higher than that in the serum of healthy adults (P<0.001) or patients with nonmalignant hepatopathy (P<0.01), and it can be detected in 40-53% of HCC patients and 33% of HCC patients with seronegative for both AFP and DCP[16-18]. Some clinical researches have indicated that the simultaneous determination of GPC3 and AFP could significantly increase the sensitivity in the diagnosis of HCC[16]. Furthermore, it has been shown that soluble GPC3 (sGPC3), the NH2-terminal portion of GPC3, is superior to AFP in the sensitivity of detecting well or moderately differentiated HCC, and the simultaneous determination of both markers improves overall sensitivity from 50% to 72%[17]. Thus, it can be seen that GPC3 could be a good supplementary to AFP in the detection. Some other investigators have reported that GPC3 mRNA is upregulated significantly in tumor tissues of HCC compared to paraneoplastic tissues of HCC, liver tissues of healthy adults and liver tissues of patients with nonmalignant hepatopathy, thus it could also be a good molecular marker for HCC[16,19-21].

ENZYMES AND ISOENZYMES

Gamma-glutamyl transferase

Serum gamma-glutamyl transferase (GGT) in healthy adults is mainly secreted by hepatic Kupffer cell and endothelial cell of bile duct, and its activity increases obviously in tissues of HCC and fetal liver. Total GGT can be divided into 13 isoenzymes (Ⅰ, Ⅰ', Ⅱ, Ⅱ', β, δ, ε, φA, Ⅶ B, φC, γA, γB) by using polymeracrylamide gradient gel electrophoresis, and some of them (Ⅰ', Ⅱ, Ⅱ') can only be detected in the serum of HCC patients. Sensitivities of GGTII have been reported to be 74.0% in detecting HCC and 43.8% in detecting small HCC[22]. Furthermore, the simultaneous determination of GGTII, DCP, and AFP can significantly improve the sensitivity over AFP alone[22]. It should be a valuable tumor marker in detecting small HCC and a good supplementary to AFP in the diagnosis of HCC.

Alpha-l-fucosidase

Alpha-l-fucosidase (AFU) is a sort of enzyme to hydrolyze fucose glycosidic linkages of glycoprotein and glycolipids. Its activity increases obviously in the serum of HCC patients (1 418.62 ± 575.76 nmol/mL per hour) compared with that in the serum of healthy adults (504.18 ± 121.88 nmol/mL per hour, P <0.05), patients with cirrhosis (831.25 ± 261.13 nmol/mL per hour), and patients with chronic hepatitis (717.71 ± 205.86 nmol/mL per hour)[23,24]. It has been reported that the sensitivity and specificity of AFU at the cut-off value of 870 nmol/(mL per h) are 81.7% and 70.7%, respectively, in contrast with 39.1% and 99.3% of AFP at the cut-off value of 400 ng/mL, and the

simultaneous determination of both markers can improve the sensitivity to 82.6%[23]. This indicates that AFU could serve as a valuable supplementary to AFP in the detection. Furthermore, it has been indicated that HCC will develop within a few years in 82% of patients with liver cirrhosis, if their serum AFU activity exceeds 700 nmol/(mL per h), and the activity of AFU is already elevated in 85% of patients at least 6 months before the detection of HCC by ultrasonography[24]. Thus, it can be seen that AFU could be a good tumor marker in detecting HCC at the earlier period.

Des-gamma-carboxyprothrombin

DCP, also known as a protein induced by vitamin K absence or antagonist II (PIVKA-II), is an abnormal product from liver carboxylation disturbance during the formation of thrombogen, and acts as an autologous mitogen for HCC cell lines[25]. Its mean serum concentration, which is not correlated to serum levels of AFP, is obviously elevated in HCC patients compared with that in healthy adults and patients with nonmalignant hepatopathy[26,27]. Though a few researches have an opposite result[28], serum and tissues DCP have been proved to be more useful than AFP in differentiating HCC from nonmalignant hepatopathy and in detecting patients with small HCC[6,26,27,29]. Cui *et al*[26] reported that the sensitivity and specificity of serum DCP (at the most commonly used cut-off value of 40 mAU/mL) in discriminating HCC from cirrhosis were 51.7% and 86.7%, respectively, which were much better than those of AFP at the cut-off value of 20 ng/mL, and 36.84% of patients with small HCC had serum DCP values above this level. Marrero *et al* [27] reported that the sensitivity and specificity of serum DCP (at the cut-off value of 125 mAU/mL) in discriminating HCC from nonmalignant hepatopathy were 89% and 86.7%, respectively, which were much better than those of AFP at the cut-off value of 11 ng/mL. Furthermore, the simultaneous determination of DCP and other tumor markers, such as AFP and AFP-L3, may have a greater accuracy than the determination of each of them alone[26,30-32]. It has been reported that the electrochemiluminescence enables measurement of low-concentration of DCP (high-sensitive DCP) in the serum, and the simultaneous determination of high-sensitive DCP (at the cut-off value of 40 mAU/mL), AFP (at the cut -off value of 20 ng/mL), and AFP-L3 (at the cut -off value of 10%) gives the highest accuracy (sensitivity of 82.1%, specificity of 82.4%, and accuracy of 82.2%)[32]. Besides the purpose of screening HCC, serum DCP could also be used as a clinicopathological or prognostic indicator for HCC patients, and may be more useful than AFP in reflecting the invasive characteristics of HCC[28,33,34]. It has been reported that patients with DCP seropositive and AFP seronegative have a higher frequency of HCC with a distinct margin, large nodule more than 3 cm, few nodules, and moderately to poorly differentiation[33,34]. Moreover, the simultaneous determination of serum DCP levels and tissue DCP expression is more valuable than either factor alone in predicting the prognosis of HCC patients[35].

GENES

Alpha-fetoprotein mRNA

Though a few researches have an opposite result[36], HCC cells spread into blood circulation and become the source of recurrence after operation, which may be the primary reason for the unsatisfactory long-term survival after surgery, and the presence of circulating HCC cells may be indicative of metastasis if AFP mRNA is detected in peripheral blood[37]. A large number of clinical researches have indicated that serum AFP mRNA detected by reverse-transcription polymerase chain reaction (RT-PCR) may be a valuable indicator of poor prognosis for HCC patients, and its expression is correlated with portal thrombosis, nodules of tumor, tumor diameter, and TNM stage ($P<0.05$)[38-42]. The recurrence-free interval of HCC patients with postoperative serum AFP mRNA positivity has been reported to be significantly shorter than that of HCC patients with postoperative negativity (53% *vs* 88% at 1 year, 37% *vs* 60% at 2 years, $P=0.014$)[42], and the recurrence-free survival rates of HCC patients with postoperative serum AFP mRNA positivity have been reported to be significantly lower than those of HCC patients with preoperative positivity (52.6% *vs* 81.8% at 1 year, 15.6% *vs* 54.5% at 2 years, and 0% *vs* 29.2% at 3 years, $P<0.001$)[43]. From the result of meta-analysis, the expression of AFP mRNA one week after surgery has also been showed to be correlated with the recurrence of HCC[44]. Moreover, the simultaneous determination of AFP mRNA and melanoma antigen gene (MAGE-1) mRNA may have a higher sensitivity and specificity[41].

Gamma-glutamyl transferase mRNA

GGT mRNA can be detected in the serum and liver tissues of healthy adults or patients with HCC, nonmalignant hepatopathy, hepatic benign tumor, and secondary carcinoma of liver. It can be divided into three types: fetal liver (type A), HepG2 cells (type B), and placenta (type C). Type A is predominant in normal liver tissues or liver tissues with nonmalignant hepatopathy, benign tumor, and secondary carcinoma ($P<0.05$). On the contrary, type B is predominant in cancerous tissues of HCC ($P<0.05$)[45-48]. During the development of HCC, the expression of GGT mRNA in liver tissues may shift from type A to type B[48]. It has been indicated that HCC patients with positive type B would have a worse outcome, earlier recurrence, and more post-recurrence death ($P=0.0107$)[49]. Therefore, the expression of tissues type B may be a valuable indicator of poor prognosis for HCC patients. The same as in liver tissues, the serum levels of type B have also been reported to be significantly higher in HCC patients than in healthy adults ($P<0.05$)[46]. Therefore, serum type B may be an available supplementary to AFP in the diagnosis of HCC.

Human telomerase reverse transcriptase mRNA

Human telomerase reverse transcriptase (hTERT) mRNA has been reported to be detectable in the serum of patients with breast cancer. Furthermore, it has also been demonstrated to be a novel and available marker for HCC diagnosis. The expression of hTERT mRNA in the serum

of HCC patients is significantly higher than that in the serum of healthy adults or patients with nonmalignant hepatopathy[50, 51], and the use of newly developed real-time quantitative reverse transcription polymerase chain reaction may improve the effectiveness of determination[50]. It has been reported that the sensitivity and specificity of hTERT mRNA in detecting HCC are 88.2% and 70.0%, respectively, which excel those of conventional tumor markers, such as AFP mRNA, AFP and DCP[50]. Moreover, it has been indicated that the expression of serum hTERT mRNA, which is associated with the serum concentration of AFP, tumor size, and tumor differentiation degree ($P < 0.001$, each), may be a valuable indicator of poor prognosis for HCC patients[50, 51].

There are some other markers in this category, which could be used as diagnostic or prognostic indicators for HCC. It has been reported that the simultaneous determination of p53 antigen and anti-p53 antibodies has a sensitivity of 41.1% in the diagnosis of HCC[52], and the over-expression of p53 in the serum or liver tissues of HCC patients prefigures the poorer prognosis and a shorter survival time ($P = 0.0014$)[52-56]. HCC patients with positive MAGE-1 or MAGE-3 mRNA die earlier because of metastasis or recurrence[57]. The sensitivity and specificity of serum human cervical cancer oncogene (HCCR) at the cut-off value of 15 μg/mL in detecting HCC are 78.2% and 95.7%, respectively. Moreover, its sensitivities could achieve 76.9% in detecting HCC patients with seronegative for AFP and 69.2% in detecting HCC patients with tumor size less than 2 cm[58].

CYTOKINES

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a secreted homodimeric cytokine that positively regulates tumor neovascularization[59]. Recent researches have suggested that angiogenesis is essential in tumor growth and progression, including that of HCC, which are typically characterized by a high level of vascularization[60-62]. In fact, it has been shown that the expressions of VEGF in cancerous tissues of HCC and HCC with microscopic venous invasion are significantly higher than that in normal liver tissues and HCC without microscopic venous invasion ($P < 0.05$), and HCC patients with over-expression of VEGF have a lower survival rate ($P < 0.05$)[63, 64]. Platelets have been reported to act as transporters of tumor-originated VEGF. It has been indicated that serum VEGF per platelet count, as an indirect theoretical estimate of VEGF in platelets, in HCC patients is significantly higher than that in healthy adults and patients with nonmalignant hepatopathy ($P < 0.01$), and the high serum VEGF per platelet count (>1.4 pg/10^6) is associated with advanced stage of HCC, portal vein thrombosis, poor response to treatment, and shorter overall survival ($P < 0.01$)[65]. Therefore, it may be an available diagnostic or prognostic indicator for HCC.

Interleukin- 8

Interleukin-8 (IL-8) is a multifunctional CXC chemokine that affects human neutrophil functions, including chemotaxis, enzyme release, and expression of surface adhesion molecules. It has direct effects on tumor and vascular endothelial cell proliferation, angiogenesis, and tumor migration. Recent researches have indicated that IL-8 regulates tumor cell growth and metastasis in liver[66]. It has been reported that the preoperative serum IL-8 levels in HCC patients are significantly elevated compared with those in healthy adults (17.6 pg/mL *vs* 1.0 pg/mL, $P = 0.046$), and its high serum levels correlate with a large tumor size (> 5 cm), absence of tumor capsule, presence of venous invasion, advanced pathological tumor-node-metastasis stage, and a poorer disease-free survival[67]. Therefore, it may be an available diagnostic or prognostic indicator for HCC.

Transforming growth factor-beta 1

Transforming growth factor-beta1 (TGF-β1) is a negative growth factor which correlates with cellular immunosuppression during the progression of HCC, and its serum levels in HCC patients have been shown to be obviously elevated compared with those in healthy adults and patients with nonmalignant hepatopathy ($P < 0.0001$)[68,69]. At the cut-off value of 800 pg/mL, the specificity of serum TGF-β1 in detecting HCC has been reported to be over 95% which is similar to AFP at the cut-off value of 200 ng/mL, but the sensitivity of serum TGF-β1 is 68% which excels AFP with the sensitivity of 24%[68]. Moreover, the elevated serum TGF-β1 can be detected in 23% of HCC patients with normal serum AFP values[69]. These researches have indicated that TGF-β1 may be a good supplementary to AFP in the diagnosis of HCC.

Tumor-specific growth factor

Malignant tumor can release tumor-specific growth factor (TSGF), which results in blood capillary amplification surrounding the tumor, into peripheral blood during its growing period. Therefore, the serum levels of TSGF can reflect the existence of tumor. It has been indicated that TSGF can be used as a diagnostic marker in detecting HCC, and its sensitivity can reach 82 % at the cut-off value of 62 U/mL[70]. Furthermore, the simultaneous determination of TSGF and other tumor markers has been shown to give a higher accuracy. It has been reported that the simultaneous determinations of TSGF, AFP, CEA, TSA, and serum ferritin have a sensitivity of 97.5%[70], and the simultaneous determinations of TSGF (at the cut-off value of 65 U/mL), AFP (at the cut-off value of 25 ng/mL) and serum ferritin (at the cut-off value of 240 μg/mL) have a sensitivity of 98.4% and specificity of 99%[71].

There are some other markers, which could be used as diagnostic or prognostic indicators for HCC, in this category. It has been reported that the determination of serum insulin-like growth factor-II (IGF-II) (at the cut-off value of 4.1 mg/g, prealbumin) has a sensitivity of 63%, specificity of 90%, and accuracy of 70% in the diagnosis of small HCC. Moreover, the simultaneous determination of IGF-II and AFP (at the cut-off value of 50 ng/mL) can improve the sensitivity to 80% and accuracy to 88%[72]. The over-expression of granulin-epithelin precursor (GEP) in cancerous tissues of HCC is associated with venous infiltration and early intrahepatic recurrence ($P < 0.05$)[73].

CONCLUSION

Serum AFP is the most widely studied screening test for detecting HCC. The normal range for serum AFP levels is 10-20 ng/mL and a level > 400 ng/mL is usually regarded as diagnostic. Furthermore, some reports have indicated that the high serum concentration of AFP correlates with the poor prognosis of HCC patients. However, two thirds of HCC patients with the nodule less than 4 cm have serum AFP levels less than 200 ng/mL and up to 20% HCC patients do not produce AFP[74]. Moreover, it has limited utility of differentiating HCC from benign hepatic disorders for the high false-positive and false-negative rates. Serum AFP-L3 and DCP are also widely used as tumor markers for HCC, and have been indicated to be more valuable than AFP in differentiating HCC from nonmalignant hepatopathy, detecting small HCC, and predicting the prognosis. Considering the large population with cirrhosis and chronic hepatitis in our country, AFP-L3 and DCP may be more useful than AFP in the diagnosis of HCC. hTERT mRNA and HCCR have been shown to have a higher accuracy than AFP in detecting HCC, but there are not enough researches to manifest their superiority. Therefore, they may not be the first choice in the detection of HCC. IGF-II has been reported to be more valuable than AFP in the diagnosis of small HCC, however, more studies are needed to demonstrate its superiority. There are some serum markers, such as GPC3, GGT II, AFU, TGF-β1, and TSGF, that have been indicated to be available supplementaries to AFP and DCP in the detection of HCC, and some of them even can be detected in HCC patients with seronegative for both AFP and DCP, the simultaneous determination of these markers may improve the accuracy. Serum AFP mRNA, which has been shown to be correlated with the metastasis and recurrence of HCC, may be the most useful marker to prefigure the prognosis of HCC patients. Some other markers, such as p53, MAGE-1, MAGE-3, GGT mRNA, VEGF, GEP, and IL-8, have also been indicated to be able to serve as prognostic indicators of HCC patients, the simultaneous determination of AFP and these markers may discover the recurrence of HCC at earlier period. In addition, there are some tumor markers, such as CYFRA 21-1[75], activin-A[76] and proliferating cell nuclear antigen[54,77,78], which do not belong to each of the categories above, but they can also be used as prognostic or screening indicators for HCC patients, especially when combined with AFP.

In a word, AFP, AFP-L3 and DCP are the most useful serum tumor markers for the detection of HCC, and the simultaneous determination of these markers could improve the accuracy, especially in differentiating HCC from nonmalignant hepatopathy. Other tumor markers, which have been mentioned in our review, could be used as supplementaries to AFP and DCP in the diagnosis of HCC, but each of them has no satisfactory accuracy in detecting HCC or prefiguring the prognosis when used alone.

REFERENCES

1 **Li L**, Zhang S, Lu F. [Research on characteristics of mortality spectrum and type composition of malignant tumors in China]. *Zhonghua Zhong Liu Za Zhi* 1997; **19**: 323-328

2 **Soresi M**, Magliarisi C, Campagna P, Leto G, Bonfissuto G, Riili A, Carroccio A, Sesti R, Tripi S, Montalto G. Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. *Anticancer Res* 2003; **23**: 1747-1753

3 **Taketa K**, Okada S, Win N, Hlaing NK, Wind KM. Evaluation of tumor markers for the detection of hepatocellular carcinoma in Yangon General Hospital, Myanmar. *Acta Med Okayama* 2002; **56**: 317-320

4 **Tan CK**, Law NM, Ng HS, Machin D. Simple clinical prognostic model for hepatocellular carcinoma in developing countries and its validation. *J Clin Oncol* 2003; **21**: 2294-2298

5 **Tangkijvanich P**, Anukulkarnkusol N, Suwangool P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Clinical characteristics and prognosis of hepatocellular carcinoma: analysis based on serum alpha-fetoprotein levels. *J Clin Gastroenterol* 2000; **31**: 302-308

6 **Fujioka M**, Nakashima Y, Nakashima O, Kojiro M. Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. *Hepatology* 2001; **34**: 1128-1134

7 **Iida H**, Honda M, Kawai HF, Yamashita T, Shirota Y, Wang BC, Miao H, Kaneko S. Ephrin-A1 expression contributes to the malignant characteristics of {alpha}-fetoprotein producing hepatocellular carcinoma. *Gut* 2005; **54**: 843-851

8 **Li MS**, Ma QL, Chen Q, Liu XH, Li PF, Du GG, Li G. Alpha-fetoprotein triggers hepatoma cells escaping from immune surveillance through altering the expression of Fas/FasL and tumor necrosis factor related apoptosis-inducing ligand and its receptor of lymphocytes and liver cancer cells. *World J Gastroenterol* 2005; **11**: 2564-2569

9 **Bae JS**, Park SJ, Park KB, Paik SY, Ryu JK, Choi CK, Hwang TJ. Acute exacerbation of hepatitis in liver cirrhosis with very high levels of alpha-fetoprotein but no occurrence of hepatocellular carcinoma. *Korean J Intern Med* 2005; **20**: 80-85

10 **Gupta S**, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50

11 **Nguyen MH**, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. *Hepatology* 2002; **36**: 410-417

12 **Khien VV**, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K, Satomura S. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001; **16**: 105-111

13 **Oka H,** Saito A, Ito K, Kumada T, Satomura S, Kasugai H, Osaki Y, Seki T, Kudo M, Tanaka M. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. *J Gastroenterol Hepatol* 2001; **16**: 1378-1383

14 **Yamashiki N**, Seki T, Wakabayashi M, Nakagawa T, Imamura M, Tamai T, Nishimura A, Inoue K, Okamura A, Arita S, Harada K. Usefulness of Lens culinaris agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3) as a marker of distant metastasis from hepatocellular carcinoma. *Oncol Rep* 1999; **6**: 1229-1232

15 **Okuda H**, Nakanishi T, Takatsu K, Saito A, Hayashi N, Yamamoto M, Takasaki K, Nakano M. Clinicopathologic features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein-L3 and seronegative for des-gamma-carboxy prothrombin in comparison with those seropositive for des-gamma-carboxy prothrombin alone. *J Gastroenterol Hepatol* 2002; **17**: 772-778

16 **Capurro M**, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; **125**: 89-97

17 **Hippo Y**, Watanabe K, Watanabe A, Midorikawa Y,

Yamamoto S, Ihara S, Tokita S, Iwanari H, Ito Y, Nakano K, Nezu J, Tsunoda H, Yoshino T, Ohizumi I, Tsuchiya M, Ohnishi S, Makuuchi M, Hamakubo T, Kodama T, Aburatani H. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. *Cancer Res* 2004; **64**: 2418-2423

18 **Nakatsura T**, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, Hosaka S, Beppu T, Ishiko T, Kamohara H, Ashihara H, Katagiri T, Furukawa Y, Fujiyama S, Ogawa M, Nakamura Y, Nishimura Y. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003; **306**: 16-25

19 **Midorikawa Y**, Ishikawa S, Iwanari H, Imamura T, Sakamoto H, Miyazono K, Kodama T, Makuuchi M, Aburatani H. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. *Int J Cancer* 2003; **103**: 455-465

20 **Sung YK**, Hwang SY, Park MK, Farooq M, Han IS, Bae HI, Kim JC, Kim M. Glypican-3 is overexpressed in human hepatocellular carcinoma. *Cancer Sci* 2003; **94**: 259-262

21 **Lü ZL**, Luo DZ, Wen JM. Expression and significance of tumor-related genes in HCC. *World J Gastroenterol* 2005; **11**: 3850-3854

22 **Cui R**, He J, Zhang F, Wang B, Ding H, Shen H, Li Y, Chen X. Diagnostic value of protein induced by vitamin K absence (PIVKAII) and hepatoma-specific band of serum gamma-glutamyl transferase (GGTII) as hepatocellular carcinoma markers complementary to alpha-fetoprotein. *Br J Cancer* 2003; **88**: 1878-1882

23 **Tangkijvanich P**, Tosukhowong P, Bunyongyod P, Lertmaharit S, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in Thailand. *Southeast Asian J Trop Med Public Health* 1999; **30**: 110-114

24 **Ishizuka H**, Nakayama T, Matsuoka S, Gotoh I, Ogawa M, Suzuki K, Tanaka N, Tsubaki K, Ohkubo H, Arakawa Y, Okano T. Prediction of the development of hepato-cellular-carcinoma in patients with liver cirrhosis by the serial determinations of serum alpha-L-fucosidase activity. *Intern Med* 1999; **38**: 927-931

25 **Suzuki M**, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, Koike K, Takaki A, Shiratori Y. Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. *J Biol Chem* 2005; **280**: 6409-6415

26 **Cui R,** Wang B, Ding H, Shen H, Li Y, Chen X. Usefulness of determining a protein induced by vitamin K absence in detection of hepatocellular carcinoma. *Chin Med J (Engl)* 2002; **115**: 42-45

27 **Marrero JA**, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003; **37**: 1114-1121

28 **Gotoh M**, Nakatani T, Masuda T, Mizuguchi Y, Sakamoto M, Tsuchiya R, Kato H, Furuta K. Prediction of invasive activities in hepatocellular carcinomas with special reference to alpha-fetoprotein and des-gamma-carboxyprothrombin. *Jpn J Clin Oncol* 2003; **33**: 522-526

29 **Miskad UA**, Yano Y, Nakaji M, Kishi S, Itoh H, Kim SR, Ku Y, Kuroda Y, Hayashi Y. Histological study of PIVKA-II expression in hepatocellular carcinoma and adenomatous hyperplasia. *Pathol Int* 2001; **51**: 916-922

30 **Okuda H**, Nakanishi T, Takatsu K, Saito A, Hayashi N, Takasaki K, Takenami K, Yamamoto M, Nakano M. Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. *Cancer* 2000; **88**: 544-549

31 **Ando E**, Tanaka M, Yamashita F, Kuromatsu R, Takada A, Fukumori K, Yano Y, Sumie S, Okuda K, Kumashiro R, Sata M. Diagnostic clues for recurrent hepatocellular carcinoma: comparison of tumour markers and imaging studies. *Eur J Gastroenterol Hepatol* 2003; **15**: 641-648

32 **Shimizu A**, Shiraki K, Ito T, Sugimoto K, Sakai T, Ohmori S, Murata K, Takase K, Tameda Y, Nakano T. Sequential fluctuation pattern of serum des-gamma-carboxy prothrombin levels detected by high-sensitive electrochemiluminescence system as an early predictive marker for hepatocellular carcinoma in patients with cirrhosis. *Int J Mol Med* 2002; **9**: 245-250

33 **Okuda H**, Nakanishi T, Takatsu K, Saito A, Hayashi N, Yamamoto M, Takasaki K, Nakano M. Comparison of clinicopathological features of patients with hepatocellular carcinoma seropositive for alpha-fetoprotein alone and those seropositive for des-gamma-carboxy prothrombin alone. *J Gastroenterol Hepatol* 2001; **16**: 1290-1296

34 **Hamamura K**, Shiratori Y, Shiina S, Imamura M, Obi S, Sato S, Yoshida H, Omata M. Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. *Cancer* 2000; **88**: 1557-1564

35 **Tang W**, Kokudo N, Sugawara Y, Guo Q, Imamura H, Sano K, Karako H, Qu X, Nakata M, Makuuchi M. Des-gamma-carboxyprothrombin expression in cancer and/or non-cancer liver tissues: association with survival of patients with resectable hepatocellular carcinoma. *Oncol Rep* 2005; **13**: 25-30

36 **Witzigmann H**, Geissler F, Benedix F, Thiery J, Uhlmann D, Tannapfel A, Wittekind C, Hauss J. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein messenger RNA in patients with hepatocellular carcinoma. *Surgery* 2002; **131**: 34-43

37 **Chen XP**, Zhao H, Zhao XP. Alternation of AFP-mRNA level detected in blood circulation during liver resection for HCC and its significance. *World J Gastroenterol* 2002; **8**: 818-821

38 **Minata M**, Nishida N, Komeda T, Azechi H, Katsuma H, Nishimura T, Kuno M, Ito T, Yamamoto Y, Ikai I, Yamaoka Y, Fukuda Y, Nakao K. Postoperative detection of alpha-fetoprotein mRNA in blood as a predictor for metastatic recurrence of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2001; **16**: 445-451

39 **Wong IH**, Yeo W, Leung T, Lau WY, Johnson PJ. Circulating tumor cell mRNAs in peripheral blood from hepatocellular carcinoma patients under radiotherapy, surgical resection or chemotherapy: a quantitative evaluation. *Cancer Lett* 2001; **167**: 183-191

40 **Jiang YF**, Yang ZH, Hu JQ. Recurrence or metastasis of HCC: predictors, early detection and experimental antiangiogenic therapy. *World J Gastroenterol* 2000; **6**: 61-65

41 **Yang SZ**, Dong JH, Li K, Zhang Y, Zhu J. Detection of AFPmRNA and melanoma antigen gene-1mRNA as markers of disseminated hepatocellular carcinoma cells in blood. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 227-233

42 **Ijichi M**, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology* 2002; **35**: 853-860

43 **Jeng KS,** Sheen IS, Tsai YC. Circulating messenger RNA of alpha-fetoprotein: a possible risk factor of recurrence after resection of hepatocellular carcinoma. *Arch Surg* 2004; **139**: 1055-1060

44 **Ding X**, Yang LY, Huang GW, Yang JQ, Liu HL, Wang W, Peng JX, Yang JQ, Tao YM, Chang ZG, Ling XS. Role of AFP mRNA expression in peripheral blood as a predictor for postsurgical recurrence of hepatocellular carcinoma: a systematic review and meta-analysis. *World J Gastroenterol* 2005; **11**: 2656-2661

45 **Han G,** Qin C. [Determination and the significance of three types of GGT mRNA in human liver tissues]. Zhonghua Gan Zang Bing Za Zhi 2002; **10**: 126-128

46 **Han GQ**, Qin CY, Shu RH. The analysis of gamma-glutamyl transpeptidase gene in different type liver tissues. *World J Gastroenterol* 2003; **9**: 276-280

47 **Han GQ,** Qin CY, Ren WH, Shi J, Wang YJ, Liu HL. Clinical impact of gamma-glutamyl transpeptidase messenger RNA subtypes on early diagnosis of hepatocellular carcinoma. *Ai Zheng* 2002; **21**: 192-195

48 **Tsutsumi M**, Sakamuro D, Takada A, Zang SC, Furukawa T, Taniguchi N. Detection of a unique gamma-glutamyl

transpeptidase messenger RNA species closely related to the development of hepatocellular carcinoma in humans: a new candidate for early diagnosis of hepatocellular carcinoma. *Hepatology* 1996; **23**: 1093-1097

49 **Sheen IS**, Jeng KS, Tsai YC. Is the expression of gamma-glutamyl transpeptidase messenger RNA an indicator of biological behavior in recurrent hepatocellular carcinoma? *World J Gastroenterol* 2003; **9**: 468-473

50 **Miura N,** Maeda Y, Kanbe T, Yazama H, Takeda Y, Sato R, Tsukamoto T, Sato E, Marumoto A, Harada T, Sano A, Kishimoto Y, Hirooka Y, Murawaki Y, Hasegawa J, Shiota G. Serum human telomerase reverse transcriptase messenger RNA as a novel tumor marker for hepatocellular carcinoma. *Clin Cancer Res* 2005; **11**: 3205-3209

51 **Miura N,** Shiota G, Nakagawa T, Maeda Y, Sano A, Marumoto A, Kishimoto Y, Murawaki Y, Hasegawa J. Sensitive detection of human telomerase reverse transcriptase mRNA in the serum of patients with hepatocellular carcinoma. *Oncology* 2003; **64**: 430-434

52 **Charuruks N**, Tangkijvanich P, Voravud N, Chatsantikul R, Theamboonlers A, Poovorawan Y. Clinical significance of p53 antigen and anti-p53 antibodies in the sera of hepatocellular carcinoma patients. *J Gastroenterol* 2001; **36**: 830-836

53 **Hu TH**, Huang CC, Lin PR, Chang HW, Ger LP, Lin YW, Changchien CS, Lee CM, Tai MH. Expression and prognostic role of tumor suppressor gene PTEN/MMAC1/TEP1 in hepatocellular carcinoma. *Cancer* 2003; **97**: 1929-1940

54 **Jing Z**, Nan KJ, Hu ML. Cell proliferation, apoptosis and the related regulators p27, p53 expression in hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 1910-1916

55 **Gianni S**, Cecchetto A, Altavilla G, Ragazzi R, Bertazzo M, De Giorgio M, Baldan A, Fagiuoli S, Farinati F. Tumour staging, morphology and p53 overexpression concur in predicting survival in hepatocellular carcinoma. *J Intern Med* 2005; **257**: 367-373

56 **Schöniger-Hekele M**, Hänel S, Wrba F, Müller C. Hepatocellular carcinoma--survival and clinical characteristics in relation to various histologic molecular markers in Western patients. *Liver Int* 2005; **25**: 62-69

57 **Mou DC**, Cai SL, Peng JR, Wang Y, Chen HS, Pang XW, Leng XS, Chen WF. Evaluation of MAGE-1 and MAGE-3 as tumour-specific markers to detect blood dissemination of hepatocellular carcinoma cells. *Br J Cancer* 2002; **86**: 110-116

58 **Yoon SK**, Lim NK, Ha SA, Park YG, Choi JY, Chung KW, Sun HS, Choi MJ, Chung J, Wands JR, Kim JW. The human cervical cancer oncogene protein is a biomarker for human hepatocellular carcinoma. *Cancer Res* 2004; **64**: 5434-5441

59 **Sugimachi K,** Tanaka S, Terashi T, Taguchi K, Rikimaru T, Sugimachi K. The mechanisms of angiogenesis in hepatocellular carcinoma: angiogenic switch during tumor progression. *Surgery* 2002; **131**: S135-S141

60 **Aoun E**, Taher A. The clinical implications of angiogenesis in the treatment of cancer. *J Med Liban* 2002; **50**: 32-38

61 **Moon WS**, Rhyu KH, Kang MJ, Lee DG, Yu HC, Yeum JH, Koh GY, Tarnawski AS. Overexpression of VEGF and angiopoietin 2: a key to high vascularity of hepatocellular carcinoma? *Mod Pathol* 2003; **16**: 552-557

62 **Zachary I**. Vascular endothelial growth factor and anti-angiogenic peptides as therapeutic and investigational molecules. *IDrugs* 2003; **6**: 224-231

63 **Liu Z,** Yan L, Xiang T, Jiang L, Yang B. Expression of vascular endothelial growth factor and matrix metalloproteinase-2 correlates with the invasion and metastasis of hepatocellular carcinoma. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 2003;**20**:249-50, 254

64 **Huang GW**, Yang LY, Lu WQ. Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. *World J Gastroenterol* 2005; **11**: 1705-1708

65 **Kim SJ,** Choi IK, Park KH, Yoon SY, Oh SC, Seo JH, Choi CW, Kim BS, Shin SW, Kim YH, Kim JS. Serum vascular endothelial growth factor per platelet count in hepatocellular carcinoma: correlations with clinical parameters and survival. *Jpn J Clin Oncol* 2004; **34**: 184-190

66 **Akiba J**, Yano H, Ogasawara S, Higaki K, Kojiro M. Expression and function of interleukin-8 in human hepatocellular carcinoma. *Int J Oncol* 2001; **18**: 257-264

67 **Ren Y**, Poon RT, Tsui HT, Chen WH, Li Z, Lau C, Yu WC, Fan ST. Interleukin-8 serum levels in patients with hepatocellular carcinoma: correlations with clinicopathological features and prognosis. *Clin Cancer Res* 2003; **9**: 5996-6001

68 **Song BC**, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, Shin JW, Lee HC, Lee YS, Suh DJ. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. *Cancer* 2002; **94**: 175-180

69 **Sacco R**, Leuci D, Tortorella C, Fiore G, Marinosci F, Schiraldi O, Antonaci S. Transforming growth factor beta1 and soluble Fas serum levels in hepatocellular carcinoma. *Cytokine* 2000; **12**: 811-814

70 **Zhu JH**, Qiu DW, Xia JR, Cheng YT. Diagnostic value of TSGF and combined tumor marker determination in patients with malignant tumors. *Chongqing Yike Daxue Xuebao* 2004; **29**: 219-220, 244

71 **Pan L**, Lei JI, Pan BI, Kong FL, Lin M, Liu SQ, Xiang XH. Significance of detection of 3 serum tumor markers in the diagnosis of primary hepatocellular carcinoma. *Zhongguo Zhongliu Linchuang Yu Kangfu* 2004; **11**: 401-402

72 **Tsai JF**, Jeng JE, Chuang LY, You HL, Wang LY, Hsieh MY, Chen SC, Chuang WL, Lin ZY, Yu ML, Dai CY. Serum insulin-like growth factor-II as a serologic marker of small hepatocellular carcinoma. *Scand J Gastroenterol* 2005; **40**: 68-75

73 **Cheung ST**, Wong SY, Leung KL, Chen X, So S, Ng IO, Fan ST. Granulin-epithelin precursor overexpression promotes growth and invasion of hepatocellular carcinoma. *Clin Cancer Res* 2004; **10**: 7629-7636

74 **Alpert E**. Human alpha-1 fetoprotein. In: Okuda K, Peters RL, eds. *Hepatocellular Carcinoma*. New York: Wiley, 1976: 353-367

75 **Ding SJ,** Li Y, Tan YX, Jiang MR, Tian B, Liu YK, Shao XX, Ye SL, Wu JR, Zeng R, Wang HY, Tang ZY, Xia QC. From proteomic analysis to clinical significance: overexpression of cytokeratin 19 correlates with hepatocellular carcinoma metastasis. *Mol Cell Proteomics* 2004; **3**: 73-81

76 **Pirisi M**, Fabris C, Luisi S, Santuz M, Toniutto P, Vitulli D, Federico E, Del Forno M, Mattiuzzo M, Branca B, Petraglia F. Evaluation of circulating activin-A as a serum marker of hepatocellular carcinoma. *Cancer Detect Prev* 2000; **24**: 150-155

77 **Shen LJ**, Zhang HX, Zhang ZJ, Li JY, Chen MQ, Yang WB, Huang R. Detection of HBV, PCNA and GST-pi in hepatocellular carcinoma and chronic liver diseases. *World J Gastroenterol* 2003; **9**: 459-462

78 **Zeng WJ**, Liu GY, Xu J, Zhou XD, Zhang YE, Zhang N. Pathological characteristics, PCNA labeling index and DNA index in prognostic evaluation of patients with moderately differentiated hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 1040-1044

S- Editor Wang J **L- Editor** Kumar M **E- Editor** Liu WF