World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v21.i37.10573

World J Gastroenterol 2015 October 7; 21(37): 10573-10583
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2015 Advances in Hepatocellular Carcinoma

Biomarkers for the early diagnosis of hepatocellular carcinoma

Nobuhiro Tsuchiya, Yu Sawada, Itaru Endo, Keigo Saito, Yasushi Uemura, Tetsuya Nakatsura

Nobuhiro Tsuchiya, Yu Sawada, Itaru Endo, Department of Gastroenterological Surgery, Graduate School of Medicine, Yokohama City University, Yokohama 236-0027, Japan

Nobuhiro Tsuchiya, Keigo Saito, Yasushi Uemura, Tetsuya Nakatsura, Division of Cancer Immunotherapy, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Kashiwa 277-8577, Japan

Author contributions: Tsuchiya N and Nakatsura T drafted the manuscript; Sawada Y, Endo I, Saito K and Uemura Y revised the manuscript.

Conflict-of-interest statement: The authors have no conflicts of interest or financial ties to disclose.

Correspondence to: Tetsuya Nakatsura, MD, PhD, Chief, Division of Cancer Immunotherapy, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, 6-5-1 Kashiwanoha, Kashiwa 277-8577, Japan. tnakatsu@east.ncc.go.jp
Telephone: +81-4-71315490
Fax: +81-4-71336606

Received: March 17, 2015
Peer-review started: March 18, 2015
First decision: April 23, 2015
Revised: May 21, 2015
Accepted: August 31, 2015
Article in press: August 31, 2015
Published online: October 7, 2015

Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide. Although the prognosis of patients with HCC is generally poor, the 5-year survival rate is > 70% if patients are diagnosed at an early stage. However, early diagnosis of HCC is complicated by the coexistence of inflammation and cirrhosis. Thus, novel biomarkers for the early diagnosis of HCC are required. Currently, the diagnosis of HCC without pathological correlation is achieved by analyzing serum α-fetoprotein levels combined with imaging techniques. Advances in genomics and proteomics platforms and biomarker assay techniques over the last decade have resulted in the identification of numerous novel biomarkers and have improved the diagnosis of HCC. The most promising biomarkers, such as glypican-3, osteopontin, Golgi protein-73 and nucleic acids including microRNAs, are most likely to become clinically validated in the near future. These biomarkers are not only useful for early diagnosis of HCC, but also provide insight into the mechanisms driving oncogenesis. In addition, such molecular insight creates the basis for the development of potentially more effective treatment strategies. In this article, we provide an overview of the biomarkers that are currently used for the early diagnosis of HCC.

Key words: α-fetoprotein; α-fetoprotein-L3; Biomarker; Des-γ-carboxyprothrombin; Glypican-3; Golgi protein-73; Hepatocellular carcinoma; MicroRNAs; Osteopontin; Squamous cell carcinoma antigen

Core tip: Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of

cancer-related death worldwide. The poor prognosis of HCC is due to the fact that diagnosis is often made at a late stage in disease development. Thus, the identification of biomarkers for diagnosis at an early stage may result in significant benefits. An up-to-date review of biomarkers that are currently used for the early diagnosis of HCC is provided in this article.

Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y, Nakatsura T. Biomarkers for the early diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2015; 21(37): 10573-10583 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i37/10573.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i37.10573

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related deaths. The number of deaths per year for HCC worldwide is similar to the incidence, with nearly 748300 new cases and 695900 deaths per year. HCC most often develops in patients with a history of cirrhosis due to chronic alcohol abuse, non-alcoholic fatty liver disease, or hepatitis C virus (HCV) infection. Continuous cycles of inflammation and healing in hepatocytes are thought to be the underlying cause of the development of HCC. However, the coexistence of inflammation and cirrhosis complicates the early diagnosis of HCC. Therefore, biomarkers that distinguish HCC from inflammation and cirrhosis are desperately needed in order to enhance prognosis of these patients. Furthermore, such biomarkers may influence the development of novel chemopreventive strategies for use during HCC surveillance of patients with cirrhosis.

Contributing to the poor prognosis of HCC is the lack of specific symptoms in the early stages of the disease. More than 60% of patients are diagnosed with late-stage disease after metastasis has occurred[1], resulting in an overall 5-year survival rate of < 16%[2]. In contrast, patients diagnosed with early stage disease have a relatively good prognosis, with a 5-year survival rate of > 70%. In patients diagnosed with early stage HCC, such as Barcelona Clinic Liver Cancer (BCLC) stage 0 and A, the 5-year survival rate with surgical intervention was > 93%[4]. Thus, detection of HCC at an early stage significantly impacts curative treatment regimens.

In Japan, early stage HCC nodules have been detected in more than 60% of patients, due to the routine practice of screening for HCC among high-risk patients[3]. The diagnosis of HCC without a pathological diagnosis can be achieved by assessing serum α-fetoprotein (AFP) levels and diagnostic imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI)[5]. Unfortunately, even this approach is inadequate, and very few HCC biomarkers demonstrate sufficient diagnostic performance for early stage HCC in clinical practice.

The ideal HCC biomarker is one that enables clinicians to diagnose asymptomatic patients and can be widely used in a screening process. In general, a biomarker valuable for clinical use achieves a level of sensitivity and specificity of ⩾ 90%, and is non-invasive and cost-effective to allow widespread use. The most desirable biomarker is therefore tumor-specific and easily detectable in bodily fluids, such as serum, plasma, and bile.

To establish a formal framework to guide biomarker evaluation and development, a five-phase program was adopted by the Early Detection Research Network (EDRN) of the National Cancer Institute (Table 1)[6]. Most markers have been evaluated in phase Ⅱ studies to evaluate their ability to detect early stage HCC, and most, with the exception of AFP, are undergoing further assessment in phase Ⅲ studies. Further studies with larger sample sizes in multiple clinical centers are needed to confirm that marker-based surveillance reduces morbidity and mortality from HCC. The present review summarizes the various biomarkers that are currently used for early diagnosis of HCC.

LIST OF HCC BIOMARKERS

AFP

AFP has been considered to be the most useful biomarker for HCC evaluation, ever since it was discovered in the serum of HCC patients in 1964[7]. In addition, it is the only biomarker that has been evaluated in a randomized controlled trial[8]. AFP is a glycoprotein with a molecular weight of about 70 kDa that transports a variety of molecules, including bilirubin, fatty acids, retinoid, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin, and possibly various drugs[9]. It is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations, the gastrointestinal tract[10]. Serum AFP reaches a maximal concentration of 3 g/L at weeks 12 to 16 of fetal life. Protein levels subsequently decrease rapidly, and thereafter only trace amounts are normally detected in serum[11]. Abnormally high serum AFP concentrations have been correlated with the development of several malignant diseases, most notably HCC[12,13]. Previously, we reported that through multivariate analysis, a minimum postoperative AFP level was determined to be a significant independent risk factor for recurrence after curative hepatectomy ($P < 0.001$)[14].

A systematic review evaluating AFP (at a threshold level of 20 ng/mL) in cirrhotic patients showed sensitivities and specificities of 41% to 65% and 80% to 94%, respectively, for HCC at any stage[15]. However, at this threshold, early stage HCC was detected in only one-third of patients with the disease[16]. The problem with AFP as a reliable HCC biomarker is that HCC is positive for the protein in only 60%-80% of cases, and false-positives make it difficult to distinguish

Table 1 Phases of biomarker validation for early cancer detection

Phase of biomarker validation	Type of study	Aim	Biomarker
Phase Ⅰ	Preclinical exploratory	Identify promising markers	
Phase Ⅱ	Case-control	Clinical assay to detect HCC	AFP-L3, DCP, GPC3, OPN, GP73, SCCA, annexin A2 suPAR, MDK, AXL, TRX, nucleic acids, miRNA
Phase Ⅲ	Retrospective longitudinal	Characterize ability of biomarker to detect HCC before it becomes clinical	
Phase Ⅳ	Prospective screening	Identify extent and characteristics of sensitivity and specificity	
Phase Ⅴ	Randomized control	Determine if biomarker screening can reduce mortality in target population	AFP

Table 2 Early diagnostic values of hepatocellular carcinoma serum biomarkers

Biomarker	Sensitivity, % (95%CI)	Specificity, % (95%CI)	Ref.
AFP	53 (46-59)	90 (87-93)	[17]
AFP-L3	28 (22-34)	97 (93-100)	[17]
DCP	61 (55-68)	70 (65-74)	[17]
GPC3	55.1 (47.9-66.2)	97.0 (95.2-98.2)	[42]
OPN	75 (58-93)	62 (51-73)	[50]
GP73	62	88	[56]
miRNA panel[1]	82.5	83.5	[101]

[1]Including miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801.

early stage HCC from other disorders, such as acute hepatitis and cirrhosis, as well as embryonic tumors and certain gastrointestinal tumors. Thus, a lower threshold may be an effective solution for early stage detection. A multicenter case-control study of patients (n = 836; HCC, n = 419 and cirrhosis, n = 417) revealed that AFP exhibited sensitivity and specificity as high as 66% and 82%, respectively, for early stage HCC (BCLC stages 0 and A) at a lower threshold of 10.9 ng/mL (Table 2)[17]. However, the sensitivity of AFP is only 53% at the commonly used cut-off of 20 ng/mL. Further research may help to optimize the threshold for AFP. However, in order to significantly improve the diagnostic accuracy for HCC, additional biomarkers are needed to complement AFP, especially due to the fact that many patients with benign liver diseases, such as chronic hepatitis, liver cirrhosis and gastrointestinal cancer, also have elevated serum AFP.

AFP-L3

AFP exists as three glycoforms, each with different binding capability to lectin *Lens culinaris* agglutinin (LCA): AFP-L1 (non-binding fraction), AFP-L2 (weak binding fraction), and AFP-L3 (binding fraction). AFP-L1 is increased in chronic hepatitis and liver cirrhosis, whereas AFP-L3 is specifically increased in HCC. Because AFP-L3 is derived only from cancer cells, it has been considered a more specific biomarker for HCC[18,19]. The concentration of AFP-L3 correlates well with AFP levels, and thus, AFP-L3 has been suggested as a biomarker for early HCC detection, due to its higher specificity than AFP[20]. For the detection of HCC, AFP-L3 is currently used at a threshold value of 10%. A large multicenter prospective study reported a specificity approaching 92%, but a sensitivity of only about 37% at this threshold for HCC at any stage[21]. Another multicenter case-control study found that AFP-L3 displayed a specificity of 97% and a sensitivity of 28% for early stage HCC diagnosis (BCLC stages 0 and A)[17]. Thus, the low sensitivity of AFP-L3 limits its potential as an HCC biomarker, even though specificity is extremely high. Moreover, because AFP-L3 is typically not detected when AFP levels are < 20 ng/mL, AFP-L3 is not relevant for the diagnosis of HCC in individuals with a total AFP concentration of < 20 ng/mL. Thus, the sensitivity for AFP-L3 appears to be adversely affected by the total AFP concentration.

Recent technical advances in higher sensitivity analytical methods with novel and advanced microfluidics-based separation science have improved the sensitivity of the AFP-L3 immunoassay[22]. The automated immunoassay for AFP-L3 is referred to as "highly sensitive AFP-L3" (hs-AFP-L3). A case-control study of hs-AFP-L3 included patients with benign liver disease (n = 74), such as chronic hepatitis and cirrhosis, as well as patients with HCC (n = 94). The study compared the performance of conventional AFP-L3 with hs-AFP-L3 and reported that hs-AFP-L3 yielded levels that were significantly higher than conventional AFP-L3, even in patients with single or small (< 20 mm in diameter) HCC nodules/tumors. The sensitivity and specificity of hs-AFP-L3 *vs* conventional AFP-L3 were 57.0% and 63.5%, and 40.4% and 81.1%, respectively[23]. These results indicate that hs-AFP-L3% could be a valuable biomarker for detecting early stage HCC and may be used for clinical practice in the near future.

Des-γ-carboxyprothrombin

Prothrombin induced by vitamin K absence Ⅱ (PIVKA Ⅱ), known as Des-γ-carboxyprothrombin (DCP), is an abnormal prothrombin molecule that is increased in HCC. During the process of malignant transformation in hepatocytes, the vitamin K-dependent carboxylase

system becomes impaired[24-27]. It is in fact a defect in posttranslational carboxylation that leads to the production of DCP[28]. In this process, DCP loses its normal prothrombin function but may take on an important role promoting malignant proliferation in HCC. Many studies have shown that the level of serum DCP in patients with benign and malignant liver diseases deviates significantly from normal, and that its diagnostic sensitivity may be greater than AFP. However, this result remains controversial[29]. In a test to screen for HCC, when compared to cases of cirrhosis and chronic hepatitis, DCP yielded a sensitivity of 72.7% and a specificity of 90.0%, a result that was comparable to AFP[25]. Because AFP and DCP are not strictly correlated, i.e. DCP is more specific to HCC and has less tendency to be elevated in other chronic liver diseases, the combination of these markers significantly improves HCC detection, yielding a sensitivity and a specificity of 74.2% and 87.2%, respectively[30].

Although DCP has demonstrated some potential as a serum biomarker for the early diagnosis of HCC, the possibility requires further investigation particularly in combination with AFP. In a large multicenter case-control study, DCP alone exhibited a sensitivity of 56% in early stage patients[31]. However, the combination of DCP with AFP increased sensitivity from 65% to 87% at 3 mo before HCC diagnosis; however, the specificity decreased from 84% to 69%.

Further studies are clearly needed to better assess the effectiveness of this combination of markers in HCC diagnosis. Moreover, DCP has been mainly examined in Asian countries, and experience with DCP in Western countries, particularly Europe, remains limited. Recently, a case-control study to compare the performances of AFP and DCP serum levels for the diagnosis of early stage HCC (BCLC stage A) was conducted in France[32]. This study included cirrhotic controls (n = 43) as well as cases with HCC (n = 85), a subset of which (n = 32) harbored early stage HCC. DCP (at a threshold value of 42 mAU/mL) performed better than AFP (at a threshold value of 5.5 ng/mL) for early stage HCC diagnosis [area under the curve (AUC) = 0.81, 95%CI: 0.697-0.924 *vs* AUC = 0.582, 95%CI: 0.443-0.722], with a sensitivity of 77% *vs* 61%, a specificity of 82% *vs* 50%, a positive predictive value (PPV) of 76% *vs* 51%, and a negative predictive value (NPV) of 83% *vs* 62%, respectively. Thus, the combination of DCP and AFP slightly improved the performance of early stage HCC diagnosis in this French cohort (AUC = 0.826, 95%CI: 0.722-0.929). These results further support the value of DCP as a biomarker in the diagnosis of early stage HCC.

Glypican-3

Glypican-3 (GPC3) belongs to the glypican family of heparan sulfate proteoglycans. It is linked to the cell membrane by a glycosyl-phosphatidylinositol anchor[33]. GPC3 is involved in cell proliferation, survival, and tumor suppression, but is normally absent in healthy and non-malignant hepatocytes. Interestingly, GPC3 appears to function differently in diverse cancers; while GPC3 is downregulated in breast cancer, ovarian cancer, and lung adenocarcinoma, it is upregulated in HCC[34,35] where it is thought to stimulate growth by upregulating autocrine/paracrine canonical Wnt signaling[36]. It has been reported that GPC3 could be detected in as many as 53% of HCC patients[37], and in our own study it was detected in 40% of HCC patients and 33% of HCC patients seronegative for both AFP and DCP[38].

As GPC3 is detected in HCC cells but not in benign liver tissues, it has potential as a biomarker for the diagnosis of early stage HCC[39,40]. Importantly, GPC3 expression appears to be independent of tumor size, as GPC3 exhibited a sensitivity of 56% in patients with early stage tumors that are < 3 cm in size[41]. In a meta-analysis, the pooled sensitivity and specificity of serum GPC3 for the diagnosis of HCC overall were 55.2% (52.9%-57.4%) and 84.2% (82.2-86.0%), respectively[42]. More specifically, GPC3 was assessed in the diagnosis of early stage HCC (BCLC 0 and A or TNM stage Ⅰ), and the observed pooled sensitivity and specificity of serum GPC3 were 55.1% (47.9%-66.2%) and 97.0% (95.2%-98.2%), respectively. For comparison, the pooled sensitivity and specificity of AFP for the same study were 34.7% (26.2%-44.1%) and 87.6% (82.6%-91.6%), respectively. Finally, the combination of GPC3 and AFP was evaluated in this study and found to increase sensitivity to 76% for early stage tumors < 3 cm in size.

The value of GPC3 is not limited to its potential as a serum biomarker. Since GPC3 is uniquely upregulated in HCC, the utility of GPC3 as an immune specific target for cancer immunotherapy has also been tested[43-45]. Measurable immune responses and antitumor efficacy with good tolerance were shown in a phase Ⅰ clinical trial of a GPC3 peptide vaccine for patients with advanced HCC[43].

Osteopontin

Osteopontin (OPN), also known as the transformation-related protein phosphatase, is an integrin-binding glycophosphoprotein that is overexpressed in many different types of malignancies, including lung, breast and colon cancer. The protein has been found to play a role in many physiological cellular functions, including migration, invasion, and metastasis[46]. One of its more critical roles has been suggested to be in the metastatic potential of various cancers[47]. Normally, OPN is expressed in bile duct epithelium, stellate cells, and Kupffer cells, but not in hepatocytes[48]. However, elevated expression of serum OPN has been reported in HCC patients compared to normal liver patients or those with liver cirrhosis or chronic hepatitis[49,50]. In a meta-analysis, the pooled sensitivity and specificity of

OPN were both at 86% for all stages of HCC[51]. In the study performed by Shang *et al*[50], OPN, at a threshold of 91 ng/mL, exhibited an increased sensitivity relative to AFP (74% *vs* 53%, respectively) when testing for HCC in a cohort including a total of 312 healthy adults and patients with cirrhosis, chronic hepatitis, and HCC. When threshold values of osteopontin at 156 ng/mL and AFP at 20 ng/mL were combined, sensitivity and specificity were even greater (95% and 96%, respectively).

Importantly, this study also investigated the utility of OPN in early diagnosis. For OPN, the AUC for discriminating between early stage HCC (BCLC stage A) and cirrhosis was 0.73. OPN demonstrated a sensitivity and specificity of 75% and 62% for early stage HCC, compared to 46% and 93% for AFP. When combined with AFP, the AUC increased to 0.81. At a threshold of 91 ng/mL for OPN, the combined use of the biomarkers resulted in a sensitivity of 83% and a specificity of 63%. Based on such findings, the value of OPN for diagnosis of early stage HCC is being further investigated in retrospective longitudinal biomarker studies.

Golgi protein-73

Golgi protein-73 (GP73) is a type Ⅱ Golgi-specific membrane protein that is normally expressed in epithelial cells of various human tissue types, but not hepatocytes. However, GP73 is detected in the serum of patients with liver disease, particularly HCC[52]. A case-control study demonstrated that serum GP73 in patients with HCC was significantly higher than in healthy adults and hepatitis B virus (HBV) carriers without hepatic disease[53]. The sensitivity and specificity of serum GP73 for HCC were 74.6% (95%CI: 71.5%-77.6%) and 97.4% (95%CI: 96.8-98.3%), respectively, compared to 58.2% and 85.3%, respectively for AFP. The combination of GP73 and AFP increased the sensitivity and specificity to 89.2% (95%CI: 86.7-91.5%) and 85.2% (95%CI: 83.4%-86.4%), respectively, with an AUC of 0.96. The combined use of GP73 and AFP-L3 for the diagnosis of low serum AFP HCC cases also demonstrated higher sensitivity (94.0%), specificity (93.1%), and better accuracy (93.3%) than either alone[54].

Investigators have considered GP73 as a potential biomarker also for early diagnosis. Serum GP73 levels showed enhanced sensitivity relative to AFP in the detection of early stage HCC[55]. In the study of Marrero *et al*[56], the sensitivity and specificity of GP73 for early HCC (United Network of Organ Sharing (UNOS) modified TNM stages 1 and 2) were similar (62% and 88%, respectively). Although these studies demonstrated that the sensitivity of GP73 was higher than that of AFP in the diagnosis of early stage HCC, whether the potential clinical value of GP73 as a serum biomarker exceeds that of AFP remains controversial. Regardless, as the elevation of serum GP73 remains moderate in virus carriers and patients with cirrhosis, GP73 should still be investigated as a potential biomarker for the diagnosis of early HCC in these patients.

Squamous cell carcinoma antigen

Squamous cell carcinoma antigen (SCCA) is a member of the high molecular weight family of serine protease inhibitors that are found in squamous epithelium and isolated from cervical carcinoma. SCCA is highly expressed in epithelial tumors and has a role in protecting tumor cells from apoptosis[57]. As SCCA is expressed as a consequence of dedifferentiation, it has been considered as a potential marker for HCC. Giannelli *et al*[58] evaluated SCCA levels in a cohort of patients (n = 210; HCC, n = 120 and cirrhosis, n = 90) and reported that HCC patients exhibited higher SCCA serum levels than cirrhotic patients. SCCA had a sensitivity of 84.2%, but the specificity was 48.9%. Subsequently, the diagnostic accuracy of SCCA was investigated taking into account only smaller HCC nodules (< 3 cm) and comparing to cirrhosis[58]. The sensitivity and specificity of SCCA were 56.1% and 74.9%, respectively, with an AUC of 0.7 (95%CI: 66%-74%) at a threshold value of 3.2 ng/mL.

SCCA expression was also tested as an immunohistochemical marker for the diagnosis of HCC. Guido *et al*[59] found that the expression of SCCA in HCC and dysplastic nodules was much higher than in regenerative nodules, indicating that the expression of SCCA had already increased early in the development of HCC. Overall, the high sensitivity and low specificity of SCCA were complementary to AFP, rendering SCCA a valuable supplementary marker for the diagnosis of HCC.

An alternative potential biomarker is the variant IgM immune complex that SCCA has been observed to form with IgM (SCCA-IgM IC) when its expression increased in the early phase of hepatocarcinogenesis. SCCA-IgM IC achieved a higher diagnostic performance than determination of the free biomarker, and furthermore was undetectable in the serum of a healthy adult. However, the detection rates of SCCA-IgM IC were 18%, 26%, and 70% in chronic hepatitis, cirrhosis, and HCC respectively[60]. The sensitivity and specificity of SCCA-IgM determination for HCC were thus 89% and 50%, respectively, with an AUC of 0.66[61]. Although the AUC was lower than that of the other discussed biomarkers, SCCA-IgM IC was consistently increased in patients with cirrhosis progressing towards HCC development, and sensitivity was higher than AFP[62]. Thus, SCCA-IgM IC may be a valuable serum marker for early HCC detection in some cases.

Annexin A2

Annexin A2 is a calcium-dependent, phospholipid-binding protein found on the surface of endothelial cells and most epithelial cells[63,64]. It is upregulated in

many tumor types and has multiple roles in various tumorigenic processes, including angiogenesis, proliferation, apoptosis, cell migration, invasion and adhesion processes, which are essential for cancer metastasis[65-68]. In HCC, serum concentrations of annexin A2 were found to be frequently elevated compared to healthy controls and individuals with benign liver disease or other malignant tumors[69-71]. Sun *et al*[72] also observed increased concentrations of annexin A2 in 83.2% of early stage HCC (BCLC stages 0 and A) and 78.4% of AFP-negative HCC patients. Annexin A2 (at 17.3 ng/μL) demonstrated sensitivity and specificity of 83.2% and 67.5%, respectively in the detection of early stage HCC, and those of AFP (15.64 ng/mL) were 54.7% and 81.3%, respectively. Moreover, the AUC of annexin A2 alone (0.79, 95%CI: 73%-85%) was greater than for AFP alone (0.73, 95%CI: 66%-80%). The combination of annexin A2 and AFP however further improved sensitivity and specificity (87.4% and 68.3%, respectively). Thus, annexin A2 might be an important independent and discriminative serological candidate biomarker for detecting early stage HCC in patients with normal serum AFP.

Soluble urokinase plasminogen activator receptor

Soluble urokinase plasminogen activator receptor (suPAR) is the circulating form of the glycosylphosphatidylinositol-linked membrane protein, urokinase-type plasminogen activator receptor (uPAR). suPAR was recently established as a biomarker for the level of activation of the immune system and cancer metastasis. suPAR serum levels are elevated in patients with ovarian cancer, colon cancer, and HCC[73-75]. A prospective study was conducted on patients (n = 267) with benign liver disease but no signs of HCC on imaging over the course of 7 years in order to determine whether serum suPAR would be a valuable molecular tool for the prediction of the future development of HCC[73]. This study revealed that within the subgroup of the high-risk European Association for the Study of Liver (EASL), a suPAR concentration of > 9.56 ng/mL yielded sensitivity of 76.0%, specificity of 90.4%, and positive and negative predictive values of 54.3% and 96.2%, respectively, for the eventual development of HCC. Based on these results, suPAR has potential as an early predictor to evaluate the risk of the development of HCC.

Midkine

Midkine (MDK) is a heparin-binding growth factor, initially identified as a retinoic acid responsive gene, which plays a critical role in cell growth, survival, migration, angiogenesis, and carcinogenesis[76]. In a study performed on patients newly diagnosed with HCC, MDK levels were found to be higher in cases of HCC than cirrhosis (0.625 ng/mL *vs* 0.15 ng/mL, $P < 0.001$) or healthy controls (0.625 ng/mL *vs* 0.125 ng/mL, $P < 0.001$)[77]. The AUC was at 0.941 (95%CI: 0.890-0.992), and for AFP at 0.671 (95%CI: 0.546-0.796) ($P < 0.001$). The sensitivity of MDK (0.387 ng/mL) to discriminate patients with early HCC (BCLC 0 and A) from those with cirrhosis was 90%, which was significantly higher than AFP (20 ng/mL) at 40%.

AXL

AXL is a receptor tyrosine kinase that has been implicated in the proliferation, survival and chemoresistance of many malignancies, including lung, breast, ovarian, colon and pancreatic cancers[78-82]. AXL is activated by the binding with growth arrest-specific protein 6 to the extracellular domain and undergoes proteolytic processing that results in the release of an 80 kDa soluble form that can be detected in serum[83]. Increased AXL expression has been identified as a poor prognostic factor for recurrence-free survival, as well as overall survival in colon and pancreatic cancer[80,82]. The diagnostic value of AXL in early stage diagnosis of HCC (BCLC stage 0) was analyzed in a multicenter study[84]. The sensitivity of AXL (76.9%) was found to be much higher than that of AFP (38.5%), and AXL outperformed AFP (AUC, 0.848 *vs* 0.797, respectively) in detecting early stage HCC. Finally, AXL and AFP together reached an extraordinarily high AUC (0.936) in detecting early stage HCC, with sensitivity at 80.8% and specificity at 92.3%.

Thioredoxins

Thioredoxins (TRXs) are thiol oxidoreductases that are ubiquitously expressed and involved in several biological processes such as, regulation of protein states, cellular apoptosis and proliferation, and protection against oxidative stress[85]. The expression of TRXs is increased in many neoplasms, and has been shown to correlate with prognosis, specifically in lung and colorectal carcinoma[86,87]. Li *et al*[88] reported on the potential availability of a TRX for the detection of early stage HCC (well-differentiated, < 2 cm HCC). In this study, the sensitivity and specificity of TRX (74.9% and 87.5%, respectively) were higher than for AFP (68.6% and 75.2%, respectively). Furthermore, with an AUC of 0.854, TRX outperformed AFP at an AUC of 0.720 in detecting early stage HCC. Again, when combined, TRX and AFP were more accurate in the detection of early stage HCC (AUC, 0.889; sensitivity, 81.3%; specificity, 93.4%).

Nucleic acids

Microarray technology has emerged as a powerful tool to probe nucleic acids for the identification of many clinically relevant molecular biomarkers, bringing a new dimension to disease diagnosis[89,90]. By screening expression arrays, Shi *et al*[91] identified three individual genes associated with HCC development, chemokine (C-X-C motif) receptor 2 (CXCR2), C-C chemokine

receptor type 2 (CCR2) and E1A-binding protein P400 (EP400), and determined their accuracies for detection of the disease: 82.4%, 78.4% and 65%, respectively. Combined measurements of the three gene markers increased the accuracy in the detection of early stage HCC (stages 0 and A) to 86% (sensitivity, 72%; specificity, 95%). Moreover, further improvement in the accuracy (91%; sensitivity, 86%; specificity 95%) occurred when AFP was included in the profile.

MicroRNAs

MicroRNAs (miRNAs) are endogenous, small (17-25 nucleotides), non-coding RNAs that bind to complementary sequences in 3'-untranslated regions of target mRNAs to induce their degradation. They are conserved across species, as miRNAs have been found to regulate diverse processes in worms, flies, and mammals, including humans[92]. Approximately 500 miRNA genes have been identified and found to be important components of complex functional pathways controlling important cellular processes, such as proliferation, differentiation, and apoptosis. In the development of human cancer, miRNAs have been determined to function both as oncogenes and as tumor suppressor genes[93]. Because each type of miRNA is stable and can downregulate hundreds of genes at a time, they can control large transcriptional programs that determine fundamental cellular features. Such diversity in functional roles enables miRNAs to be used as diagnostic tools for early cancer detection, risk and prognosis assessment, and as new therapeutic targets[94].

miRNAs associated with HCC development have been investigated as biomarkers to diagnose the disease. Some of these miRNAs have been shown to accurately predict poor prognosis in HCC[95]. For example, studies have indicated that miR 200a and miR 200b, two members of the miR 200 family, are deregulated during the development of both HCC and liver fibrosis[96-98]. The increased levels of serum miR-21 have been used to distinguish cases of HCC from chronic hepatitis and healthy controls. In the case of HCC *vs* chronic hepatitis, the sensitivity and specificity were 61.1% and 83.3%, respectively, with an AUC of 0.773, and in the case of HCC *vs* healthy controls the values were 87.3% and 92.0%, respectively, with an AUC of 0.773. Both values were superior to that of AFP as a biomarker in HCC[99]. Serum miR-15b and miR-130b are additional potential miRNA markers that are significantly upregulated in HCC[100]. For the detection of HCC, miR-130b exhibited an AUC of 0.913 (sensitivity, 87.7%; specificity, 81.4%). In contrast, while the sensitivity of miR-15b for detecting HCC was extremely high at 98.3%, its specificity was very poor (15.3%). The high sensitivity of serum miR-15b and miR-130b as biomarkers for HCC is potentially favorable, particularly for patients with early stage HCC, who may have low AFP levels. A panel of seven miRNAs (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801) has been shown to have high diagnostic accuracy in the early diagnosis of HBV-related HCC (BCLC stage 0 and A; AUC, 0.888)[101].

A few features, in addition to their expression profiles, make miRNAs particularly attractive as potential biomarkers. First, since many dysregulated miRNAs are highly stable and readily detected in serum and plasma in HCC patients, they may more generally have high AUCs in the detection of HCC as well as any other disease state. Second, miRNAs appear in the urine, which represents a non-invasive and easily obtainable resource for biomarkers. In fact, the detection of five deregulated miRNAs (miR-625, miR-532, miR-618, miR-516-5P, and miR-650) in the urine has already been used to screen high-risk patients for the early detection of HCC[102]. The presence of miRNAs in body fluids, such as urine, may represent a gold mine of biomarkers for cancer. However, further investigation is necessary to establish specific circulating miRNA(s) as reliable and accurate in the detection of HCC at an early stage.

CONCLUSION

The diagnosis of HCC patients remains difficult, especially early in the development of the disease, and yet early and accurate diagnosis of HCC patients is vital in order to improve prognosis. Promising biomarkers for diagnosis of HCC have been successfully identified in several studies. However, current data suggest that no single biomarker alone is likely to have optimal sensitivity and specificity for the detection of HCC, particularly at early stages of development. In many studies, combinations of several biomarkers have been shown to complement each other and improve the early diagnostic rate. Incorporation of clinical variables, such as age and sex, into models based on combinations of biomarkers could further enhance the predictive performance of the models for HCC detection. More randomized controlled studies investigating such biomarkers will help to validate optimal combinations of them for successful detection of early stage HCC.

Although the emphasis in this review has been on the early detection of HCC, biomarkers have an additional exciting role in the development of personalized treatment. In this regard, any biomarker, even one with low sensitivity, has the potential to serve as an important indicator for a molecularly targeted drug. For example, GPC3-targeted immunotherapy, including a peptide vaccine and antibody, elicited some anti-tumor effect and showed good tolerance[45]. In our Phase Ⅰ clinical trial of GPC3-derived peptide vaccines, the disease control rate [partial response (PR) + stable disease (SD)] was 60.6% at two months after initiation of treatment. A median survival of 12.2 mo was observed in patients exhibiting a high frequency of GPC3-specific cytotoxic T lymphocytes (CTLs) with no severe adverse events, compared to 8.5 mo in

individuals with a low GPC3-specific CTL frequency (P = 0.033). GPC3 antibodies (GC33) had an SD of more than 26 wk in 4 of 15 (16.7%) patients[103]. The median overall survival in the group with high expression of GPC3 (49.4 wk) was greater than in the groups with low or no GPC3 expression (13.0 wk).

In conclusion, advances in technologies, such as mass spectrometry and next-generation sequencing, hold great promise for the identification of novel early diagnostic biomarkers for HCC. Circulating miRNAs are particularly intriguing as a whole new class of biomarkers and may outperform traditional serum protein markers. The added advantages are that some changes in miRNAs are detected early and in body fluids so that they can be easily monitored. However, even if any of the markers discussed perform well as biomarkers, therapeutic efficacy remains poor, especially in the absence of imaging. New treatment options and novel imaging modalities are therefore desperately needed. Finally, novel biomarkers may provide important clues to our understanding of oncogenesis, and ultimately lead to better treatment strategies. Simultaneous advancement in these many medical disciplines will hopefully initiate change in the poor prognosis of HCC patients.

REFERENCES

1 **Altekruse SF**, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009; **27**: 1485-1491 [PMID: 19224838 DOI: 10.1200/JCO.2008.20.7753]

2 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]

3 **Izumi N**. Diagnostic and treatment algorithm of the Japanese society of hepatology: a consensus-based practice guideline. *Oncology* 2010; **78** Suppl 1: 78-86 [PMID: 20616588 DOI: 10.1159/000315234]

4 **Takayama T**, Makuuchi M, Kojiro M, Lauwers GY, Adams RB, Wilson SR, Jang HJ, Charnsangavej C, Taouli B. Early hepatocellular carcinoma: pathology, imaging, and therapy. *Ann Surg Oncol* 2008; **15**: 972-978 [PMID: 18236118 DOI: 10.1245/s10434-007-9685-0]

5 **Aghoram R**, Cai P, Dickinson JA. Alpha-foetoprotein and/or liver ultrasonography for screening of hepatocellular carcinoma in patients with chronic hepatitis B. *Cochrane Database Syst Rev* 2012; **9**: CD002799 [PMID: 22972059 DOI: 10.1002/14651858.CD002799.pub2]

6 **Pepe MS**, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001; **93**: 1054-1061 [PMID: 11459866]

7 **Tatarinov IuS**. Detection of embryo-specific alpha-globulin in the blood serum of a patient with primary liver cancer. *Vopr Med Khim* 1964; **10**: 90-91 [PMID: 14207501]

8 **Zhang BH**, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130**: 417-422 [PMID: 15042359 DOI: 10.1007/s00432-004-0552-0]

9 **Mizejewski GJ**. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. *Exp Biol Med* (Maywood) 2001; **226**: 377-408 [PMID: 11393167]

10 **Gitlin D**, Perricelli A, Gitlin JD. The presence of serum alpha-fetoprotein in sharks and its synthesis by fetal gastrointestinal tract and liver. *Comp Biochem Physiol B* 1973; **46**: 207-215 [PMID: 4127981]

11 **Debruyne EN**, Delanghe JR. Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. *Clin Chim Acta* 2008; **395**: 19-26 [PMID: 18538135 DOI: 10.1016/j.cca.2008.05.010]

12 **Chayvialle JA**, Ganguli PC. Radioimmunoassay of alpha-fetoprotein in human plasma. *Lancet* 1973; **1**: 1355-1357 [PMID: 4122743]

13 **Waldmann TA**, McIntire KR. The use of a radioimmunoassay for alpha-fetoprotein in the diagnosis of malignancy. *Cancer* 1974; **34**: suppl: 1510-ppl: 1515 [PMID: 4138906]

14 **Nobuoka D**, Kato Y, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kinoshita T, Nakatsura T. Postoperative serum alpha-fetoprotein level is a useful predictor of recurrence after hepatectomy for hepatocellular carcinoma. *Oncol Rep* 2010; **24**: 521-528 [PMID: 20596642]

15 **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 [PMID: 12834318]

16 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [PMID: 11394657]

17 **Marrero JA**, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, Reddy KR, Harnois D, Llovet JM, Normolle D, Dalhgren J, Chia D, Lok AS, Wagner PD, Srivastava S, Schwartz M. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 110-118 [PMID: 19362088 DOI: 10.1053/j.gastro.2009.04.005]

18 **Spangenberg HC**, Thimme R, Blum HE. Serum markers of hepatocellular carcinoma. *Semin Liver Dis* 2006; **26**: 385-390 [PMID: 17051452 DOI: 10.1055/s-2006-951606]

19 **Sato Y**, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993; **328**: 1802-1806 [PMID: 7684823 DOI: 10.1056/NEJM199306243282502]

20 **Li D**, Mallory T, Satomura S. AFP-L3: a new generation of tumor marker for hepatocellular carcinoma. *Clin Chim Acta* 2001; **313**: 15-19 [PMID: 11694234]

21 **Sterling RK**, Jeffers L, Gordon F, Venook AP, Reddy KR, Satomura S, Kanke F, Schwartz ME, Sherman M. Utility of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxy prothrombin, alone or in combination, as biomarkers for hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2009; **7**: 104-113 [PMID: 18849011 DOI: 10.1016/j.cgh.2008.08.041]

22 **Kagebayashi C**, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, Satomura M, Kurosawa T, Watanabe M, Kawabata T, Chang W, Li C, Bousse L, Wada HG, Satomura S. Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. *Anal Biochem* 2009; **388**: 306-311 [PMID: 19250915 DOI: 10.1016/j.ab.2009.02.030]

23 **Oda K**, Ido A, Tamai T, Matsushita M, Kumagai K, Mawatari S, Saishoji A, Kure T, Ohno K, Toyokura E, Imanaka D, Moriuchi A, Uto H, Oketani M, Hashiguchi T, Tsubouchi H. Highly sensitive lens culinaris agglutinin-reactive α-fetoprotein is useful for early detection of hepatocellular carcinoma in patients with chronic liver disease. *Oncol Rep* 2011; **26**: 1227-1233 [PMID: 21874252 DOI: 10.3892/or.2011.1425]

24 **Bertino G**, Ardiri AM, Boemi PM, Ierna D, Interlandi D, Caruso L, Minona E, Trovato MA, Vicari S, Li Destri G, Puleo S. A study about mechanisms of des-gamma-carboxy prothrombin's production in hepatocellular carcinoma. *Panminerva Med* 2008; **50**: 221-226 [PMID: 18927526]

25 **Carr BI**, Kanke F, Wise M, Satomura S. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. *Dig Dis Sci* 2007; **52**: 776-782 [PMID: 17253135 DOI: 10.1007/s10620-006-9541-2]

26 **Kaibori M**, Matsui Y, Yanagida H, Yokoigawa N, Kwon AH, Kamiyama Y. Positive status of alpha-fetoprotein and des-gamma-carboxy prothrombin: important prognostic factor for recurrent hepatocellular carcinoma. *World J Surg* 2004; **28**: 702-707 [PMID: 15185000 DOI: 10.1007/s00268-004-7205-y]

27 **Leerapun A**, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, Stadheim LM, Aderca I, Moser CD, Nagorney DM, LaRusso NF, de Groen PC, Menon KV, Lazaridis KN, Gores GJ, Charlton MR, Roberts RO, Therneau TM, Katzmann JA, Roberts LR. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol Hepatol* 2007; **5**: 394-402; quiz 267 [PMID: 17368240 DOI: 10.1016/j.cgh.2006.12.005]

28 **Naraki T**, Kohno N, Saito H, Fujimoto Y, Ohhira M, Morita T, Kohgo Y. gamma-Carboxyglutamic acid content of hepatocellular carcinoma-associated des-gamma-carboxy prothrombin. *Biochim Biophys Acta* 2002; **1586**: 287-298 [PMID: 11997080]

29 **Volk ML**, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. *Cancer Biomark* 2007; **3**: 79-87 [PMID: 17522429]

30 **Bertino G**, Neri S, Bruno CM, Ardiri AM, Calvagno GS, Malaguarnera M, Toro A, Malaguarnera M, Clementi S, Bertino N, Di Carlo I. Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. *Minerva Med* 2011; **102**: 363-371 [PMID: 22193346]

31 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]

32 **Poté N**, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, Puy H, Bedossa P, Paradis V. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol* 2015; **62**: 848-854 [PMID: 25450201 DOI: 10.1016/j.jhep.2014.11.005]

33 **Filmus J**. The contribution of in vivo manipulation of gene expression to the understanding of the function of glypicans. *Glycoconj J* 2002; **19**: 319-323 [PMID: 12975611 DOI: 10.1023/A:1025312819804]

34 **Filmus J**, Capurro M. The role of glypican-3 in the regulation of body size and cancer. *Cell Cycle* 2008; **7**: 2787-2790 [PMID: 18787398]

35 **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 [PMID: 12824919]

36 **Capurro MI**, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005; **65**: 6245-6254 [PMID: 16024626 DOI: 10.1158/0008-5472.CAN-04-4244]

37 **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 [PMID: 12851874]

38 **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 [PMID: 12788060]

39 **Libbrecht L**, Severi T, Cassiman D, Vander Borght S, Pirenne J, Nevens F, Verslype C, van Pelt J, Roskams T. Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006; **30**: 1405-1411 [PMID: 17063081 DOI: 10.1097/01.pas.0000213323.97294.9a]

40 **Shafizadeh N**, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol* 2008; **21**: 1011-1018 [PMID: 18536657 DOI: 10.1038/modpathol.2008.85]

41 **Tangkijvanich P**, Chanmee T, Komtong S, Mahachai V, Wisedopas N, Pothacharoen P, Kongtawelert P. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. *J Gastroenterol Hepatol* 2010; **25**: 129-137 [PMID: 19793164 DOI: 10.1111/j.1440-1746.2009.05988.x]

42 **Jia X**, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. *Arch Med Res* 2014; **45**: 580-588 [PMID: 25446613 DOI: 10.1016/j.arcmed.2014.11.002]

43 **Motomura Y**, Senju S, Nakatsura T, Matsuyoshi H, Hirata S, Monji M, Komori H, Fukuma D, Baba H, Nishimura Y. Embryonic stem cell-derived dendritic cells expressing glypican-3, a recently identified oncofetal antigen, induce protective immunity against highly metastatic mouse melanoma, B16-F10. *Cancer Res* 2006; **66**: 2414-2422 [PMID: 16489048 DOI: 10.1158/0008-5472.CAN-05-2090]

44 **Nakatsura T**, Komori H, Kubo T, Yoshitake Y, Senju S, Katagiri T, Furukawa Y, Ogawa M, Nakamura Y, Nishimura Y. Mouse homologue of a novel human oncofetal antigen, glypican-3, evokes T-cell-mediated tumor rejection without autoimmune reactions in mice. *Clin Cancer Res* 2004; **10**: 8630-8640 [PMID: 15623647 DOI: 10.1158/1078-0432.CCR-04-1177]

45 **Sawada Y**, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, Mizuno S, Ishii H, Nakachi K, Konishi M, Nakagohri T, Takahashi S, Gotohda N, Takayama T, Yamao K, Uesaka K, Furuse J, Kinoshita T, Nakatsura T. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res* 2012; **18**: 3686-3696 [PMID: 22577059 DOI: 10.1158/1078-0432.CCR-11-3044]

46 **Shevde LA**, Das S, Clark DW, Samant RS. Osteopontin: an effector and an effect of tumor metastasis. *Curr Mol Med* 2010; **10**: 71-81 [PMID: 20205680]

47 **Rangaswami H**, Bulbule A, Kundu GC. Osteopontin: role in cell signaling and cancer progression. *Trends Cell Biol* 2006; **16**: 79-87 [PMID: 16406521 DOI: 10.1016/j.tcb.2005.12.005]

48 **Kawashima R**, Mochida S, Matsui A, YouLuTuZ Y, Ishikawa K, Toshima K, Yamanobe F, Inao M, Ikeda H, Ohno A, Nagoshi S, Uede T, Fujiwara K. Expression of osteopontin in Kupffer cells and hepatic macrophages and Stellate cells in rat liver after carbon tetrachloride intoxication: a possible factor for macrophage migration into hepatic necrotic areas. *Biochem Biophys Res Commun* 1999; **256**: 527-531 [PMID: 10080931 DOI: 10.1006/bbrc.1999.0372]

49 **Abu El Makarem MA**, Abdel-Aleem A, Ali A, Saber R, Shatat M, Rahem DA, Sayed D. Diagnostic significance of plasma osteopontin in hepatitis C virus-related hepatocellular carcinoma. *Ann Hepatol* 2011; **10**: 296-305 [PMID: 21677331]

50 **Shang S**, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajrang S, Hainaut P, Marrero JA, Beretta L. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2012; **55**: 483-490 [PMID: 21953299 DOI: 10.1002/hep.24703]

51 **Wan HG**, Xu H, Gu YM, Wang H, Xu W, Zu MH. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 706-714 [PMID: 25034355 DOI: 10.1016/j.clinre.2014.06.008]

52 **Kladney RD**, Cui X, Bulla GA, Brunt EM, Fimmel CJ. Expression of GP73, a resident Golgi membrane protein, in viral and nonviral liver disease. *Hepatology* 2002; **35**: 1431-1440 [PMID: 12029628 DOI: 10.1053/jhep.2002.32525]

53 **Mao Y**, Yang H, Xu H, Lu X, Sang X, Du S, Zhao H, Chen W, Xu Y, Chi T, Yang Z, Cai J, Li H, Chen J, Zhong S, Mohanti SR, Lopez-Soler R, Millis JM, Huang J, Zhang H. Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. *Gut* 2010; **59**: 1687-1693 [PMID: 20876776 DOI: 10.1136/gut.2010.214916]

54 **Xu WJ**, Guo BL, Han YG, Shi L, Ma WS. Diagnostic value of alpha-fetoprotein-L3 and Golgi protein 73 in hepatocellular carcinomas with low AFP levels. *Tumour Biol* 2014; **35**:

12069-12074 [PMID: 25209179 DOI: 10.1007/s13277-014-2506-8]
55 **Hu JS**, Wu DW, Liang S, Miao XY. GP73, a resident Golgi glycoprotein, is sensibility and specificity for hepatocellular carcinoma of diagnosis in a hepatitis B-endemic Asian population. *Med Oncol* 2010; **27**: 339-345 [PMID: 19399652 DOI: 10.1007/s12032-009-9215-y]
56 **Marrero JA**, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, Comunale MA, D'Amelio A, Lok AS, Block TM. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. *J Hepatol* 2005; **43**: 1007-1012 [PMID: 16137783 DOI: 10.1016/j.jhep.2005.05.028]
57 **Suminami Y**, Kishi F, Sekiguchi K, Kato H. Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. *Biochem Biophys Res Commun* 1991; **181**: 51-58 [PMID: 1958219]
58 **Giannelli G**, Fransvea E, Trerotoli P, Beaugrand M, Marinosci F, Lupo L, Nkontchou G, Dentico P, Antonaci S. Clinical validation of combined serological biomarkers for improved hepatocellular carcinoma diagnosis in 961 patients. *Clin Chim Acta* 2007; **383**: 147-152 [PMID: 17582392 DOI: 10.1016/j.cca.2007.05.014]
59 **Guido M**, Roskams T, Pontisso P, Fassan M, Thung SN, Giacomelli L, Sergio A, Farinati F, Cillo U, Rugge M. Squamous cell carcinoma antigen in human liver carcinogenesis. *J Clin Pathol* 2008; **61**: 445-447 [PMID: 17893121 DOI: 10.1136/jcp.2007.051383]
60 **Beneduce L**, Castaldi F, Marino M, Quarta S, Ruvoletto M, Benvegnù L, Calabrese F, Gatta A, Pontisso P, Fassina G. Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. *Cancer* 2005; **103**: 2558-2565 [PMID: 15887222 DOI: 10.1002/cncr.21106]
61 **Pozzan C**, Cardin R, Piciocchi M, Cazzagon N, Maddalo G, Vanin V, Giacomin A, Pontisso P, Cillo U, Farinati F. Diagnostic and prognostic role of SCCA-IgM serum levels in hepatocellular carcinoma (HCC). *J Gastroenterol Hepatol* 2014; **29**: 1637-1644 [PMID: 24635038 DOI: 10.1111/jgh.12576]
62 **Pontisso P**, Quarta S, Caberlotto C, Beneduce L, Marino M, Bernardinello E, Tono N, Fassina G, Cavalletto L, Gatta A, Chemello L. Progressive increase of SCCA-IgM immune complexes in cirrhotic patients is associated with development of hepatocellular carcinoma. *Int J Cancer* 2006; **119**: 735-740 [PMID: 16550605 DOI: 10.1002/ijc.21908]
63 **Sharma MC**, Sharma M. The role of annexin II in angiogenesis and tumor progression: a potential therapeutic target. *Curr Pharm Des* 2007; **13**: 3568-3575 [PMID: 18220793]
64 **Lokman NA**, Ween MP, Oehler MK, Ricciardelli C. The role of annexin A2 in tumorigenesis and cancer progression. *Cancer Microenviron* 2011; **4**: 199-208 [PMID: 21909879 DOI: 10.1007/s12307-011-0064-9]
65 **Sharma MR**, Koltowski L, Ownbey RT, Tuszynski GP, Sharma MC. Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. *Exp Mol Pathol* 2006; **81**: 146-156 [PMID: 16643892 DOI: 10.1016/j.yexmp.2006.03.003]
66 **Shiozawa Y**, Havens AM, Jung Y, Ziegler AM, Pedersen EA, Wang J, Wang J, Lu G, Roodman GD, Loberg RD, Pienta KJ, Taichman RS. Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J Cell Biochem* 2008; **105**: 370-380 [PMID: 18636554 DOI: 10.1002/jcb.21835]
67 **Tressler RJ**, Updyke TV, Yeatman T, Nicolson GL. Extracellular annexin II is associated with divalent cation-dependent tumor cell-endothelial cell adhesion of metastatic RAW117 large-cell lymphoma cells. *J Cell Biochem* 1993; **53**: 265-276 [PMID: 8263043 DOI: 10.1002/jcb.240530311]
68 **Díaz VM**, Hurtado M, Thomson TM, Reventós J, Paciucci R. Specific interaction of tissue-type plasminogen activator (t-PA) with annexin II on the membrane of pancreatic cancer cells activates plasminogen and promotes invasion in vitro. *Gut* 2004; **53**: 993-1000 [PMID: 15194650]
69 **Hollås H**, Aukrust I, Grimmer S, Strand E, Flatmark T, Vedeler A. Annexin A2 recognises a specific region in the 3'-UTR of its cognate messenger RNA. *Biochim Biophys Acta* 2006; **1763**: 1325-1334 [PMID: 17045350 DOI: 10.1016/j.bbamcr.2006.08.043]
70 **Ji NY**, Park MY, Kang YH, Lee CI, Kim DG, Yeom YI, Jang YJ, Myung PK, Kim JW, Lee HG, Kim JW, Lee K, Song EY. Evaluation of annexin II as a potential serum marker for hepatocellular carcinoma using a developed sandwich ELISA method. *Int J Mol Med* 2009; **24**: 765-771 [PMID: 19885616]
71 **Zhao P**, Zhang W, Wang SJ, Yu XL, Tang J, Huang W, Li Y, Cui HY, Guo YS, Tavernier J, Zhang SH, Jiang JL, Chen ZN. HAb18G/CD147 promotes cell motility by regulating annexin II-activated RhoA and Rac1 signaling pathways in hepatocellular carcinoma cells. *Hepatology* 2011; **54**: 2012-2024 [PMID: 21809360 DOI: 10.1002/hep.24592]
72 **Sun Y**, Gao G, Cai J, Wang Y, Qu X, He L, Liu F, Zhang Y, Lin K, Ma S, Yang X, Qian X, Zhao X. Annexin A2 is a discriminative serological candidate in early hepatocellular carcinoma. *Carcinogenesis* 2013; **34**: 595-604 [PMID: 23188673 DOI: 10.1093/carcin/bgs372]
73 **Chounta A**, Ellinas C, Tzanetakou V, Pliarhopoulou F, Mplani V, Oikonomou A, Leventogiannis K, Giamarellos-Bourboulis EJ. Serum soluble urokinase plasminogen activator receptor as a screening test for the early diagnosis of hepatocellular carcinoma. *Liver Int* 2015; **35**: 601-607 [PMID: 25348952 DOI: 10.1111/liv.12705]
74 **Henic E**, Borgfeldt C, Christensen IJ, Casslén B, Høyer-Hansen G. Cleaved forms of the urokinase plasminogen activator receptor in plasma have diagnostic potential and predict postoperative survival in patients with ovarian cancer. *Clin Cancer Res* 2008; **14**: 5785-5793 [PMID: 18794088 DOI: 10.1158/1078-0432.CCR-08-0096]
75 **Lomholt AF**, Christensen IJ, Høyer-Hansen G, Nielsen HJ. Prognostic value of intact and cleaved forms of the urokinase plasminogen activator receptor in a retrospective study of 518 colorectal cancer patients. *Acta Oncol* 2010; **49**: 805-811 [PMID: 20524776 DOI: 10.3109/0284186X.2010.491086]
76 **Muramatsu T**. Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. *J Biochem* 2002; **132**: 359-371 [PMID: 12204104]
77 **Shaheen KY**, Abdel-Mageed AI, Safwat E, AlBreedy AM. The value of serum midkine level in diagnosis of hepatocellular carcinoma. *Int J Hepatol* 2015; **2015**: 146389 [PMID: 25737783 DOI: 10.1155/2015/146389]
78 **Byers LA**, Diao L, Wang J, Saintigny P, Girard L, Peyton M, Shen L, Fan Y, Giri U, Tumula PK, Nilsson MB, Gudikote J, Tran H, Cardnell RJ, Bearss DJ, Warner SL, Foulks JM, Kanner SB, Gandhi V, Krett N, Rosen ST, Kim ES, Herbst RS, Blumenschein GR, Lee JJ, Lippman SM, Ang KK, Mills GB, Hong WK, Weinstein JN, Wistuba II, Coombes KR, Minna JD, Heymach JV. An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. *Clin Cancer Res* 2013; **19**: 279-290 [PMID: 23091115 DOI: 10.1158/1078-0432.CCR-12-1558]
79 **D'Alfonso TM**, Hannah J, Chen Z, Liu Y, Zhou P, Shin SJ. Axl receptor tyrosine kinase expression in breast cancer. *J Clin Pathol* 2014; **67**: 690-696 [PMID: 24904064 DOI: 10.1136/jclinpath-2013-202161]
80 **Dunne PD**, McArt DG, Blayney JK, Kalimutho M, Greer S, Wang T, Srivastava S, Ong CW, Arthur K, Loughrey M, Redmond K, Longley DB, Salto-Tellez M, Johnston PG, Van Schaeybroeck S. AXL is a key regulator of inherent and chemotherapy-induced invasion and predicts a poor clinical outcome in early-stage colon cancer. *Clin Cancer Res* 2014; **20**: 164-175 [PMID: 24170546 DOI: 10.1158/1078-0432.CCR-13-1354]
81 **Rankin EB**, Fuh KC, Taylor TE, Krieg AJ, Musser M, Yuan J, Wei K, Kuo CJ, Longacre TA, Giaccia AJ. AXL is an essential factor and therapeutic target for metastatic ovarian cancer. *Cancer Res* 2010; **70**: 7570-7579 [PMID: 20858715 DOI: 10.1158/0008-5472.CAN-10-1267]
82 **Song X**, Wang H, Logsdon CD, Rashid A, Fleming JB, Abbruzzese JL, Gomez HF, Evans DB, Wang H. Overexpression of receptor tyrosine kinase Axl promotes tumor cell invasion and survival in pancreatic ductal adenocarcinoma. *Cancer* 2011; **117**: 734-743

[PMID: 20922806 DOI: 10.1002/cncr.25483]
83 **Pinato DJ**, Mauri FA, Lloyd T, Vaira V, Casadio C, Boldorini RL, Sharma R. The expression of Axl receptor tyrosine kinase influences the tumour phenotype and clinical outcome of patients with malignant pleural mesothelioma. *Br J Cancer* 2013; **108**: 621-628 [PMID: 23361052 DOI: 10.1038/bjc.2013.9]
84 **Reichl P**, Fang M, Starlinger P, Staufer K, Nenutil R, Muller P, Greplova K, Valik D, Dooley S, Brostjan C, Gruenberger T, Shen J, Man K, Trauner M, Yu J, Gao CF, Mikulits W. Multicenter analysis of soluble Axl reveals diagnostic value for very early stage hepatocellular carcinoma. *Int J Cancer* 2015; **137**: 385-394 [PMID: 25529751 DOI: 10.1002/ijc.29394]
85 **Nordberg J**, Arnér ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 2001; **31**: 1287-1312 [PMID: 11728801]
86 **Kakolyris S**, Giatromanolaki A, Koukourakis M, Powis G, Souglakos J, Sivridis E, Georgoulias V, Gatter KC, Harris AL. Thioredoxin expression is associated with lymph node status and prognosis in early operable non-small cell lung cancer. *Clin Cancer Res* 2001; **7**: 3087-3091 [PMID: 11595699]
87 **Raffel J**, Bhattacharyya AK, Gallegos A, Cui H, Einspahr JG, Alberts DS, Powis G. Increased expression of thioredoxin-1 in human colorectal cancer is associated with decreased patient survival. *J Lab Clin Med* 2003; **142**: 46-51 [PMID: 12878985 DOI: 10.1016/S0022-2143(03)00068-4]
88 **Li J**, Cheng ZJ, Liu Y, Yan ZL, Wang K, Wu D, Wan XY, Xia Y, Lau WY, Wu MC, Shen F. Serum thioredoxin is a diagnostic marker for hepatocellular carcinoma. *Oncotarget* 2015; **6**: 9551-9563 [PMID: 25871387]
89 **Villanueva A**, Minguez B, Forner A, Reig M, Llovet JM. Hepatocellular carcinoma: novel molecular approaches for diagnosis, prognosis, and therapy. *Annu Rev Med* 2010; **61**: 317-328 [PMID: 20059340 DOI: 10.1146/annurev.med.080608.100623]
90 **Marquardt JU**, Galle PR, Teufel A. Molecular diagnosis and therapy of hepatocellular carcinoma (HCC): an emerging field for advanced technologies. *J Hepatol* 2012; **56**: 267-275 [PMID: 21782758 DOI: 10.1016/j.jhep.2011.07.007]
91 **Shi M**, Chen MS, Sekar K, Tan CK, Ooi LL, Hui KM. A blood-based three-gene signature for the non-invasive detection of early human hepatocellular carcinoma. *Eur J Cancer* 2014; **50**: 928-936 [PMID: 24332572 DOI: 10.1016/j.ejca.2013.11.026]
92 **Ferracin M**, Veronese A, Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. *Expert Rev Mol Diagn* 2010; **10**: 297-308 [PMID: 20370587 DOI: 10.1586/erm.10.11]
93 **Wang J**, Sen S. MicroRNA functional network in pancreatic cancer: from biology to biomarkers of disease. *J Biosci* 2011; **36**: 481-491 [PMID: 21799259]
94 **Schütte K**, Schulz C, Link A, Malfertheiner P. Current biomarkers for hepatocellular carcinoma: Surveillance, diagnosis and prediction of prognosis. *World J Hepatol* 2015; **7**: 139-149 [PMID: 25729470 DOI: 10.4254/wjh.v7.i2.139]
95 **Han ZB**, Chen HY, Fan JW, Wu JY, Tang HM, Peng ZH. Up-regulation of microRNA-155 promotes cancer cell invasion and predicts poor survival of hepatocellular carcinoma following liver transplantation. *J Cancer Res Clin Oncol* 2012; **138**: 153-161 [PMID: 22071603 DOI: 10.1007/s00432-011-1076-z]
96 **Murakami Y**, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006; **25**: 2537-2545 [PMID: 16331254 DOI: 10.1038/sj.onc.1209283]
97 **Hung CS**, Liu HH, Liu JJ, Yeh CT, Chang TC, Wu CH, Ho YS, Wei PL, Chang YJ. MicroRNA-200a and -200b mediated hepatocellular carcinoma cell migration through the epithelial to mesenchymal transition markers. *Ann Surg Oncol* 2013; **20** Suppl 3: S360-S368 [PMID: 22868917 DOI: 10.1245/s10434-012-2482-4]
98 **Murakami Y**, Toyoda H, Tanaka M, Kuroda M, Harada Y, Matsuda F, Tajima A, Kosaka N, Ochiya T, Shimotohno K. The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. *PLoS One* 2011; **6**: e16081 [PMID: 21283674 DOI: 10.1371/journal.pone.0016081]
99 **Tomimaru Y**, Eguchi H, Nagano H, Wada H, Kobayashi S, Marubashi S, Tanemura M, Tomokuni A, Takemasa I, Umeshita K, Kanto T, Doki Y, Mori M. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 167-175 [PMID: 21749846 DOI: 10.1016/j.jhep.2011.04.026]
100 **Liu AM**, Yao TJ, Wang W, Wong KF, Lee NP, Fan ST, Poon RT, Gao C, Luk JM. Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective cohort study. *BMJ Open* 2012; **2**: e000825 [PMID: 22403344 DOI: 10.1136/bmjopen-2012-000825]
101 **Zhou J**, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781-4788 [PMID: 22105822 DOI: 10.1200/JCO.2011.38.2697]
102 **Abdalla MA**, Haj-Ahmad Y. Promising Candidate Urinary MicroRNA Biomarkers for the Early Detection of Hepatocellular Carcinoma among High-Risk Hepatitis C Virus Egyptian Patients. *J Cancer* 2012; **3**: 19-31 [PMID: 22211142]
103 **Zhu AX**, Gold PJ, El-Khoueiry AB, Abrams TA, Morikawa H, Ohishi N, Ohtomo T, Philip PA. First-in-man phase I study of GC33, a novel recombinant humanized antibody against glypican-3, in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2013; **19**: 920-928 [PMID: 23362325 DOI: 10.1158/1078-0432.CCR-12-2616]

P- Reviewer: Liu XL, Van Vlierberghe H, Wu CC, Xu WG
S- Editor: Yu J **L- Editor**: A **E- Editor**: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327