

Review Article

An update of biochemical markers of hepatocellular carcinoma

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Abstract

The definition of a tumor marker is broad, which covers a wide spectrum of biomacromolecules synthesized in excess concentration by a wide variety of neoplastic cells. Tumor markers can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained inactive in the normal cells. These markers consist of any products of either tumor itself or the host in reaction to tumor's presence that distinguishes malignant tissues from benign and is measurable in body fluids or tissues. They increase with progressive or recurrent disease, decrease with response to treatment, and normalize with remission. Clinical applications include screening in asymptomatic individuals, confirming a suspected diagnosis, assisting in tumor classification and staging, prognosis, monitoring treatment response, surveillance for residual disease, and early detection of recurrent disease.

Keywords:

HCC, Alpha-fetoprotein, AFP, Glypican-3, SCCA, GEP, Enzymes and isoenzymes.

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Introduction

Several factors influence the clinical utility of any marker. Those attributable to type of tumor include its prevalence among population under investigation in addition to the availability of an effective treatment regimen. ⁽¹⁾ Factors attributable to the test include its sensitivity (ability to detect individuals with the disease) and specificity (ability to discern individuals with the disease from those that are either normal or have some other condition). These can be calculated using the following formulas: ^(2, 3)

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

A receiver operator characteristic (ROC) analysis demonstrating the trade-off between specificity and sensitivity at various concentrations is useful in determining an appropriate cut-off value. Values that allow maximization of sensitivity, specificity, or the best trade-off between the two can be selected depending on the clinical use of the test. Optimum sensitivity is desirable for screening applications, while maximizing specificity is for confirmatory purposes. ⁽¹⁻⁴⁾ Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and now the third leading cause of cancer deaths worldwide. The incidence of HCC is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of HCC. ^(4, 5) The presentation of HCC has evolved significantly over the past few decades. While, in the past, this carcinoma generally presented at an advanced stage with right upper quadrant pain, weight loss, and signs of decompensated liver disease, HCC is now increasingly recognized at a much earlier stage as a consequence of the routine screening of patients with known cirrhosis, using cross-sectional imaging studies and serum alpha-fetoprotein measurements. ^(4, 5) There are two categories of biochemical markers that are being used or studied for the detection of HCC: ⁽⁵⁾ Oncofetal antigens and glycoprotein antigens and enzymes and isoenzymes.

A. Oncofetal antigens and glycoprotein antigens as biomarkers of HCC

Alfa-fetoprotein (α -fetoprotein, AFP) is large serum glycoprotein, belonging to onco-development protein. ⁽⁶⁾ Under physiological conditions, AFP is a fetal-specific glycoprotein with a molecular weight of 70 kD. It is synthesized primarily by the embryonic liver, by cells of vital line sac and by fetal intestinal tract in the first trimester of pregnancy, has a half-life of 5-7 days. The serum concentration of AFP declines rapidly after birth and its expression is repressed in adults. Pathologically, patients with chronic liver disease can express AFP in absence of HCC. AFP is elevated in HCC, embryonic carcinomas, and in gastric and lung cancer. ⁽⁷⁾

AFP is synthesized by approximately half of HCC, and used in differential diagnosis and follow-up of patients with liver tumors, but no correlation has been found between the clinical behavior and AFP production in HCC. ⁽⁸⁾ Serum AFP is associated with two main problems; first, the transient rise in the serum level of AFP in chronic liver disease patients especially during exacerbation of hepatitis (serum level >100 ng/ml) (low specificity), slight increases are usual in acute hepatitis, chronic hepatitis and cirrhosis. The second is that among all patients diagnosed with HCC, AFP levels may be normal in up to 40% of patients, particularly during the early stages (low sensitivity). Thus it is associated with false positive and negative rates. ⁽⁹⁾ AFP is not elevated in all patients with HCC. Factors such as age, sex, infection with HBV and HCV, cirrhosis and acute liver necrosis specially size and form of tumor pathology can influence AFP level. The test had a sensitivity of 39-65%, and a specificity of 76-94% in the presence of HCC in previously published studies. ⁽¹⁰⁾ AFP interpretation is complicated by intermittent elevations of AFP in 12-13% of patients with chronic HBV or HCV. In chronic hepatic diseases, high risk of HCC is present in patients with cirrhosis due to HBV, HCV, or alcohol abuse. While other causes of chronic hepatic injury and cirrhosis have lower risk of HCC. Although the role of AFP in the diagnosis of advanced HCC is well recognized, at least one-third of small HCC and 30% of advanced HCC will be missed unless another diagnostic

tool is used. ⁽¹¹⁾ AFP assessment and liver ultrasound can be a diagnostic factor in screening and follow up of the patients at risk of HCC. The AFP sensitivity differs on the basis of selected cut off level in many countries. Results of many studies showed that serum AFP level more than 400-500 ng/ml is specific for HCC, but its range between 20-200 ng/ml is non-specific in diagnosis. ⁽¹²⁾ According to a study, patients with serum AFP greater than 1000 ng/ml had a higher incidence of vascular invasion (61%) compared to patients with AFP level less than or equal to 1000 ng/ml (32%). This may relate to the finding that well-differentiated tumors express lower levels of AFP. ⁽¹³⁾

Lens culinaris agglutinin-reactive or AFP

There are three different AFP variants according to their binding capacity for lens culinaris agglutinin (LCA), differing in their sugar chains (AFP-L1, AFP-L2, AFP-L3). AFP-L1, the non- LCA-bound fraction, is the main glycoform of AFP in the serum of patients with non-malignant chronic liver disease. In contrast, Lens culinaris-reactive AFP, also known as AFP-L3, is the main glycoform of AFP in the serum of HCC patients and can be detected in approximately one third of patients with small HCC (< 3 cm), when cut-off values of 10% to 15% are used. Its sensitivity and specificity ranges from 75% to 96.90% and 90% to 92%, respectively at cut-off level of 15%. ⁽⁵⁾ In a study of HCC patients with lesions less than 2 cm in size, using cut-off level of 10% was diagnostic for presence of HCC. AFP-L3 was found to be associated with poorly differentiated and advanced HCC. Higher AFP-L3 levels were found in hyper vascular HCC, compared to iso- or hypo vascular HCC. ⁽¹⁴⁾ Malignant liver cells produce AFP-L3, even when HCC is at its early stages, and especially when tumor mass is supplied by hepatic artery. AFP-L3 positive HCC has potential for rapid growth and early metastasis. ⁽¹⁵⁾ Elevated levels of AFP-L3 were associated with a shorter tumor doubling time in comparison with low levels of AFP-L3. AFP-L3 acts as marker for clearance of HCC after treatment and predictor of recurrence as failure to decline to normal level indicates residual disease. Recurrence of HCC is expected when AFP-L3 levels increase to >10% or rise after normalization with treatment. ⁽¹⁶⁾ AFP-L3 levels

were found to be related to progression from moderately to poorly differentiated tumors. AFP-L3 > 15% is a predictor for HCC recurrence. HCC patients with AFP-L3 > 10% had a higher frequency of poorly differentiated tumors. Thus, this biomarker may be able to predict advanced tumor stage and a worse prognosis. ⁽¹⁷⁾ AFP-L3 level of 15% or more is correlated with HCC- associated portal vein invasion, both total serum AFP and AFP-L3 can be measured, and estimating AFP-L3/AFP ratio is helpful in diagnosis and prognosis of HCC. ⁽¹⁸⁾

Glypican-3 (GPC3)

GPC3 isoncofetal protein being one of members of heparin sulfate proteoglycans anchored to plasma membrane through glycosyl-phosphatidylinositol. It is involved in regulation of cell proliferation and survival during embryonic development and functions as a tumor suppressor. ⁽¹⁹⁾ It is composed of two subunits that are linked by one or more disulfide bonds. The two subunits are produced by cleavage of GPC3 by a convertase, which generates an amino-terminal fragment of 40 kD and a COOH-terminal fragment of 30 kD. COOH-terminal fragment of GPC3 will carry the heparan sulfate chains. ⁽²⁰⁾ GPC3 is involved in growth inhibitory functions, and that loss of GPC3 expression may contribute to uncontrolled cell growth. Alteration of GPC3 expression due to gene mutations leads to an overgrowth syndrome in humans (Simpson- Golabi- Behmel syndrome) which is characterized by pre- and postnatal overgrowth and multiple embryonic abnormalities. ⁽²¹⁾ GPC3 is absent in hepatocytes of healthy subjects and patients with nonmalignant hepatopathy, and can be detected in about 50% of HCC patients and 33% of HCC patients seronegative for AFP. ⁽²²⁾ Wang et al. detected GPC3 expression in 3 (60%) of 5 atypical hepatocellular adenomas, suggesting that GPC3 could be helpful in cases with malignant progression. ⁽²³⁾ Detection of focal GPC3 expression in cirrhotic nodules in a small subset of HCC cases, but not in cirrhotic livers or large regenerative nodules in cases without HCC. So, GPC3 may function as an early biomarker in hepatocarcinogenesis. Although high-grade dysplastic nodules tended to express GPC3 more than low-grade dysplastic nodules, no

statistically significant difference was detected. GPC3 stimulates growth of HCC cells by up regulating autocrine/ paracrine canonical Wnt signaling. GPCs stimulate canonical and non-canonical pathways. GPC3 regulates migration, adhesion, and actin cytoskeleton organization in mammary tumor cells through Wnt signaling modulation. ⁽²⁴⁾ GPC3 has been shown to bind to fibroblast growth factor 2 and may function as a co receptor for fibroblast growth factor 2. Two human heparin-degrading endosulfatases, sulfatase 1 and sulfatase 2, have been found to be involved in HCC. Sulfatase 2 up-regulates GPC3, promotes fibroblast growth factor signaling, and decreases survival in HCC. ⁽²⁵⁾

Squamous cell carcinoma antigen (SCCA)

SCCA is a serine protease inhibitor that is found in the spinous and granular layers of normal squamous epithelium, but it is expressed typically by neoplastic cells of epithelial origin. Two different isoforms, encoded by 2 highly homologous genes SCCA1 and SCCA2, have been identified as the neutral and acidic forms, respectively. ⁽²⁶⁾ SCCA1 and SCCA2 proteins are present in suprabasal layers of normal stratified squamous epithelium, whereas the acidic form, has been detected in a number of epithelial malignancies as those of cervix, lung, head and neck, and used as a clinical marker. ⁽²⁷⁾ SCCA1 and SCCA2 protect neoplastic cells from apoptotic death induced by several kinds of stimuli, and in vivo experiments have demonstrated that SCCA1 can promote tumor growth. ⁽²⁸⁾ Beale et al. found that SCCA1 was not elevated in patients with HCC arising on top of nonalcoholic and alcoholic fatty liver disease which suggests that this HCC marker may be etiology related and so results are different in Egyptian patients as HCC arise on top of HCV and/or HBV liver disease. ⁽²⁹⁾ Also, the choice of optimal biomarkers for HCC surveillance may be determined by the etiology of underlying liver disease. SCCA-1 has previously shown as an AFP complementary biomarker, in viral hepatitis related HCC. ⁽³⁰⁾ According to the European Association for the study of the liver, SCCA is diagnostic above 200 ng/ml. The concentration of circulating SCCA-IgM immune complex at different stages of liver disease reflects the extent of SCCA over expression detected by

immunohistochemistry in liver specimens. SCCA-IgM immune complex does not overlap with AFP and offers the possibility to increase the sensitivity for detecting HCC without compromising specificity. SCCA over expression may be an early event of carcinogenesis in liver, because immunohistochemical analysis in dysplastic nodules that are deemed HCC putative morphologic precursors indicates high SCCA expression, whereas benign hepatic nodules do not show SCCA expression. ⁽³¹⁾ However, no elevated levels of free SCCA in patients with HCC but high level of SCCA-IgM immune complex is detected. Analyses of human systemic immune response against malignancy have led to identification of a number of tumor-associated antigens, suggesting that at least some malignancies are immunogenic. ⁽³²⁾ In patients with HCC, free SCCA variants were not elevated compared with healthy controls or compared with free or SCCA-complexed anti SCCA IgG. In patients with HCC, initial low-dose exposure to SCCA variants may induce a weak IgM immune response without activation of IgG antibody-mediated immunity. Over expression of SCCA1 and SCCA2 has been reported in all surgically resected HCC specimens but in none of the normal control livers as detected by immunohistochemistry. The clinical value of SCCA-IgM as a predictive biomarker of HCC in patients with cirrhosis has been reported, demonstrating that the increase in serum concentrations over time of SCCA-IgM in patients with cirrhosis was associated to higher risk of development of HCC. ⁽²⁷⁾

Golgi Protein 73 (GP73)

GP73 is a 73-kD resident Golgi membrane protein of unknown function. GP73 is a type II trans membrane protein with a single, N-terminal trans membrane domain and an extensive, C-terminal coiled-coil domain located on the luminal surface of the Golgi apparatus. GP73 messenger RNA levels were increased in HCV related human liver disease. GP73 is expressed by biliary epithelial cells in normal liver. Hepatocyte expression of GP73 is up-regulated in patients with acute hepatitis, cirrhosis and HCC, while there is no difference in biliary epithelial cell expression of this marker. ⁽³³⁾ GP73 is superior to AFP for detection of early HCC in patients with

cirrhosis. Measurement of serum GP73 based on immunoblots revealed that HCC patients had higher levels than patients with cirrhosis. For diagnosis of early HCC, this marker had a higher sensitivity (62%) than AFP (25%). GP73 had a sensitivity of 69% and a specificity of 75% at the optimal cut off point of 10 relative units for HCC diagnosis.⁽³⁴⁾ Serum GP73 levels were elevated in 57% of patients with HCC associated with normal AFP levels. GP73 is up regulated in hepatitis virus-related HCC. In a study, the level of GP73 was increased compared to normal controls or colorectal cancer in immunoblot analysis. This highlighted the potential of GP73 as HCC biomarker.⁽³⁵⁾ The mechanism by which sGP73 reaches the circulation was worked out in cell culture studies. Despite its steady-state localization within the cis-Golgi complex, GP73 cycles through the distal secretory apparatus and transiently reaches the apical cell membrane, from which it returns to the Golgi complex via an endosomal retrieval pathway. A smaller form of GP73 is present in supernatants of several cell lines. This secreted form is generated by N-terminal cleavage of the molecule by the proprotein convertase furin after amino acid 55, resulting in release of large C-terminal ectodomain into extracellular space.⁽³⁶⁾ sGP73 serum levels reported by Riener et al.⁽³⁷⁾ were 80-fold higher than those reported by Gu et al.⁽³⁸⁾ with a median serum concentration in normal subjects of 4 µg/ml, which is well within the range of many classical plasma proteins. Riener et al. reported that by using tissue microarrays, GP73 was established to be over expressed in malignant liver tumors in comparison with non-cancerous tissues. The degree of GP73 expression correlated with the tumor.⁽³⁷⁾

Osteopontin(OPN)

Osteopontin, a highly phosphorylated and glycosylated calcium-binding secretory protein is expressed widely and has many functions including cell adhesion and migration, immune and inflammatory response, antiapoptosis, suppression of nitric oxide synthase, and bone calcification. OPN is arginine- glycine- aspartate containing phosphoprotein. It is involved in normal tissue remodeling processes as well as certain diseases.⁽³⁹⁾ As a proinflammatory agent, it is chemotactic for,

supports adhesion of, and modulates the function of T cells and monocytes/macrophages. OPN induces chemotaxis and haptotaxis of T cells and macrophages in vitro, functioning as a typical chemo attractant.⁽⁴⁰⁾ Osteopontin has been found to be essential for the dissemination of various cancers. Transfection of the cells with anti-sense OPN RNA reduced the malignancy of the cells and caused the decrease of tumorigenesis.⁽⁴¹⁾ Osteopontin contributes to tumorigenesis and its expression has been found in carcinomas of colon, pancreas, and multiple myeloma in human and has been shown to be a diagnostic tool. OPN is immobilized ECM molecule but also in a secreted form in body fluids including plasma. Its diagnostic sensitivity and specificity for HCC was 87% and 82%, respectively, suggesting diagnostic accuracy of OPN.⁽⁴²⁾ Osteopontin identification by immunohistochemistry in HBV-positive HCC was positively correlated with portal vein and lymph node invasion and negatively correlated with worse disease-free and overall survival.⁽⁴³⁾ HCC with OPN mRNA over expression was associated with a lower 10-year survival rate than HCC without OPN over expression.⁽⁴⁴⁾ In HCC patients, higher plasma OPN positively correlated with reduced liver function, as defined by increasing tumor stage, suggesting use of plasma OPN as a prognostic factor.⁽⁴²⁾

Asialo-alpha- acid glycoprotein (As AGP)

Serum alpha acid glycoprotein (AGP) is a heavily glycosylated plasma protein with 5 complex N-glycan, has been studied in patients with acute or chronic inflammation, as well as various different malignant diseases.⁽⁴⁵⁾ Song et al.⁽⁴⁶⁾ observed that serum alpha acid glycoprotein concentration was increased in patients with liver cirrhosis and HCC. Kim et al.⁽⁴⁷⁾ reported that measurement of serum As AGP concentration may improve the diagnosis and prognosis of hepatic disease (especially liver cirrhosis and /or HCC) and assist in monitoring the risk of progression to more severe state. The serum As AGP value was slightly increased in inactive chronic hepatitis but moderately increased in patients with active chronic hepatitis. The important finding is that serum As AGP values were only slightly increased in patients with acute hepatitis as well as those with a variety of non- hepatic

diseases compared to the outstanding increase in liver cirrhosis and HCC cases. ⁽⁴⁷⁾ The increase of serum As AGP concentration is restricted to LC or HCC cases. The sensitivity of As AGP was 88.9% and specificity was 84.2%. These results indicate that an increase in serum As AGP concentration may be positively correlated with a progression of hepatic disease to an advanced state. ⁽⁴⁷⁾

Granulin-epithelin precursor (GEP)

Granulin-epithelinprecursor (GEP, also called progranulin, or acrogranin) is a growth factor and a secretory protein that is capable of stimulating cell proliferation. Its reduced expression is associated with inhibition of tumorigenic potential. ⁽⁴⁸⁾ Strong GEP expression was associated with aggressive HCC features including large tumor size, presence of venous infiltration, and early intrahepatic recurrence. Venous infiltration is a microscopic feature that demonstrates presence of clusters of cancer cells in vascular space lined by endothelial cells, and this microscopic metastasis is predictive of intrahepatic metastasis and poor prognosis. Therefore, involvement of GEP in the aggressive HCCs suggests that GEP may play a role in HCC progression. ⁽⁴⁹⁾ Down regulating GEP using the antisense approach could significantly reduce the tumor genicity and tumor growth rate of HCC in a thymic nude mice model. These observations suggested that GEP is an attractive target for cancer therapy. However, the mode of gene delivery and infection/transfection efficiency of the antisense approach has limited its use as successful cancer-targeted therapy. GEP is important in HCC progression, so neutralizing the secreted GEP by specific monoclonal antibodies is an effective means of HCC treatment. ⁽⁴⁹⁾

P-glycoprotein (P-gp)

P-glycoprotein is a 170 kD, membrane bound glycoprotein encoded by the multidrug resistance gene and functions as an ATP-dependent pump propelling drugs out of the cell cytoplasm. Because of its involvement in drug resistance, the expression of P-gp has been studied in several neoplasms and increased P-gp expression has been found in epithelial tumors, as those of the colon and

breast. ⁽⁵⁰⁾ P-glycoprotein over expression had been demonstrated in cancer with recurrence. Most advanced HCC is insensitive to anticancer drugs which might be related to high frequency of expression of the multidrug resistance gene. P-gp expression may also be concerned with tumor progression and differentiation. ⁽⁵¹⁾ Activation of the multidrug resistance gene in hepatic tissues has been studied in animal models; rodent hepatocarcinogenesis models show that enhanced multidrug resistance expression is associated with the later stages of carcinogenesis. In cell line models, the multidrug resistance gene, which encodes P-gp, is responsive to p53 tumor suppressor gene. Mutant p53 has been shown to stimulate multidrug resistance promoter. In HCC, mutations of p53 are frequent events, particularly in areas with a high HCC incidence, a high prevalence of hepatitis B virus infection, and a high aflatoxin level in foodstuffs. ⁽⁵²⁾

Laminin

Laminin is one of the main glycoproteins of the basement membrane and participates in adhesion, migration, cellular differentiation and growth, inflammatory response and the maintenance of the cytoskeleton upon its binding to several components of the matrix, such as collagen type IX and heparan-sulphate. ⁽⁵³⁾ Laminin B1 is a diagnostic biomarker because of its overexpression in HCC. It was not only a marker for detection of early HCC, but also its expression was positively correlated with the increased number of tumor nodules, size of the tumors and advanced stage of the tumor characteristics. ⁽⁴⁹⁾ Laminin B1 is the major component of nuclear lamina which provides structural support of the nuclear envelope and is involved in controlling gene expression by organization of chromatin, DNA replication, repair and transcription. Laminin B1 controls oxidative stress via octamer transcription factor-1. Phosphorylation of laminin B1 mediated by the inositide-specific phospholipase C signaling results in cell proliferation via G2/M cell cycle progression. ⁽⁵⁴⁾ Proteolytic degradation of laminin B1 have been reported during radiation induced apoptosis. Nucleic acid in plasma may come from lysis of circulating cancer cells caused by tumor necrosis, apoptosis, or active

release. Some of the apoptotic cells would release laminin B1 mRNA into the circulation and it might be detectable in the patients' plasma. Some studies explored the possibility of using RT-PCR in measuring plasma laminin B1 mRNA in the HCC patients. ⁽⁵⁵⁾

Laminin receptors are found on the surface of a wide range of cells, such as platelets, muscle cells, neutrophils, endothelial cells and hepatocytes. The existence of a class of transmembrane receptors for laminin known as integrin's has been demonstrated. These integrin's are involved in the mechanisms of cell-cell, cell-matrix and pathogen-cell adhesion. ⁽⁵⁶⁾ Laminin binding proteins have been described in *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori*, and *Candida albicans*. With the development of anti-laminin antibodies, directed against laminin P1 portion, increased levels of this protein were observed in more advanced stages of fibrosis in patients with hepatic disease. ⁽⁵⁷⁾ Serum laminin levels in supra-hepatic veins were higher compared to those found in the renal and femoral veins of patients with fibrosis or hepatic cirrhosis, which would be indicative of its hepatic synthesis. Since an increased synthesis of basement membrane in the spleen has been reported, the reduced levels of laminin after splenectomy suggest an important participation of an extra hepatic source for the serum levels of laminin. ⁽⁵⁸⁾ Thus, not only circulating levels of laminin reflect hepatic processes of synthesis and degradation, but also increase of synthesis of basement membranes, as a result of the congestion observed in other splanchnic organs. Studies with fibroscan have found sensitivity and specificity for the diagnosis of portal hypertension in cirrhotic patients far higher than those found through the determination of serum laminin. ⁽⁵⁹⁾ Such determination was discontinued as it did not demonstrate to be superior to those of other such components of the ECM as hyaluronic acid. In studies, laminin determination has been included in a set of test together with hydroxyproline, prothrombin activity, and alanine transferase/ aspartate transferase in the diagnosis of advanced fibrosis in chronic hepatitis C. ⁽⁶⁰⁾ Serum laminin levels have been studied in patients with liver diseases and in cancer especially in cases with tumor proliferation and invasion. Serum values tend

to increase with emergence of metastases, irrespective of tumor lineage or the organ originating the neoplasm. Serum laminin could be regarded as a tumor marker in cases of alterations in the basement membrane, proliferation and tumor invasion. In fact serum laminin concentration is increased in metastatic cancer of different origins as gastric adenocarcinoma, hepatocellular carcinoma, colorectal cancer, epithelial ovarian tumor. ⁽⁶¹⁾ By using polyclonal antibodies against laminin isolated from human placenta, a significant increase in serum and ascetic laminin levels was observed in patients with peritoneal carcinomatosis when compared to patients with hepatic cirrhosis with or without HCC. ⁽⁶²⁾ Immunohistochemical studies have shown laminin in neoplastic transformation of hepatocytes and despite representation of group of patients with HCC once they presented advanced disease with large tumor masses with high serum AFP levels, blood and ascites laminin values did not distinguish these patients from those with liver cirrhosis without HCC. In benign and malignant ascites, serum laminin were higher and it showed high ability for diagnosis of malignant ascites, with 75% sensitivity, 100% specificity and 91% accuracy. ⁽⁶³⁾

Galectin-1 (Gal-1)

Galectin-1 (Gal-1) protein belongs to a family of soluble lactose-binding lectins (galectins). Gal-1 is a multifunctional protein involved in various aspects of tumorigenesis (cell-ECM and cell-cell interactions, cell migration, angiogenesis, tumor-immune escape) and has been described as a cancer target. Expression of galectin-1 has been documented in different malignant tumors including astrocytoma, melanoma and prostate, thyroid, colon, bladder and ovary carcinomas. ⁽⁶⁴⁾ Intracellular galectin-1 may play a role in the initiation of transformed phenotype of tumors. Galectin-1 interacts with oncogenic h-RAS and contributes to membrane anchorage of h-RAS. Over expression of galectin-1 in tumor cells results in an increase in both the membrane association of h-RAS and cell transformation. ⁽⁶⁵⁾

Neopterin

Neopterin is one of the intermediate metabolites in biosynthesis of

tetrahydrobiopterin, which is an essential coenzyme in the hydroxylation of aromatic amino acids from guanosinotriphosphostate. The enhanced in serum neopterin in patients with malignant tumors is related not due to the production and emission of neopterin by cancer cells themselves, but due to the chronic activation of cellular immunity responding to the presence of malignant tumors. Thus, neopterin is recognized as a chemical marker. ⁽⁶⁶⁾ Neopterin is released from monocytes/macrophages under stimulus of interferon- γ produced from activated T-cells. In addition, persistently elevated neopterin levels might reflect the degree of macrophage activation. Serum and urinary neopterin were reported to be increased in viral infections, including acquired immunodeficiency syndrome, and graft rejection. It has been used to diagnose or monitor diseases as a biological marker of increased cell-mediated immunity. It is indicator of prognosis in some malignant diseases. ⁽⁶⁷⁾ Neopterin derivatives may even promote the growth and progress of malignant tumor. Neopterin is an intermediate in the biosynthesis of tetrahydrobiopterin which plays an important role in the synthesis of neurotransmitters as catecholamine's and serotonin. The urinary excretion of neopterin is considered to be an indicator of cell proliferation and to be associated with the presence of malignant growth. ⁽⁶⁸⁾ Urinary neopterin levels parallel the tumor size in HCC patients, but no relationship with serum AFP. The enhanced excretion of neopterin may become a marker for detection of HCC and may be of use in assessment of tumor growth. HCC patients were separated into two groups according to urinary neopterin levels, the group with high urinary neopterin levels appeared to have serious hepatic dysfunction than the group with normal urinary neopterin levels so, urinary neopterin levels may be associated with abnormality of liver function tests and tumor size in HCC patients. Because of this overlap of urinary neopterin concentrations in patients with a normal range of urinary neopterin values, urinary neopterin may not be helpful in the diagnosis of tumor or distant metastases, but immune activation, reflected by increased neopterin concentrations, is an independent indicator of poor prognosis in cancer patients. ⁽⁶⁹⁾

B. Enzymes and isoenzymes as biomarkers of HCC.

Des-gamma-carboxy-prothrombin(DCP)

Desgamma-carboxy-prothrombin is abnormal prothrombin that is lacking γ -carboxy residues and so cannot become an active clotting enzyme. Although the precise causes of DCP production are unknown, the concentration of vitamin K is normal in non-tumorous parts of the liver but is decreased significantly in HCC tissues. ⁽⁷⁰⁾ DCP, also known as a protein induced by vitamin K absence or antagonist II (PIVKA-II). It is an abnormal product from liver carboxylation disturbance during formation of thrombogen, and acts as an autologous mitogen for HCC cell lines. ⁽⁷¹⁾ Vitamin K hydroquinone acts as substrate for enzyme γ -glutamyl carboxylase, which catalyzes the addition of carbon dioxide to the γ -carbon of protein-bound glutamic acid. Oxygenation of vitamin K hydroquinone provides the energy to derive the carboxylation reaction, leading to formation of γ -carboxy glutamic acid residues and vitamin K oxide. Vitamin K oxide is reduced by another reductase back to vitamin K, to enter another cycle. ⁽⁷²⁾ Prothrombin, blood coagulation factor II, is the major vitamin K dependent blood coagulation protein synthesized in the liver. In the absence of vitamin K or in the presence of vitamin K antagonists such as warfarin, vitamin K dependent gamma carboxylation in the liver is inhibited and DCP levels are increased. ⁽⁷³⁾ The γ -carboxy glutamic acid residues are able to bind and attached to phosphate groups of platelet membrane phospholipid. Much of blood clotting is a result of blood clotting proteins assembling into a complex on the membranes of platelet and endothelial cells within these complexes, the factors can efficiently contact one another to become activated and participate in clot formation. ⁽⁷⁴⁾ In exploring the structure of DCP, there are two kringle domains similar to those of hepatocyte growth factor (HGF), which was identified as a potent mitogen for mature hepatocytes. Kringle domains are mandatory for HGF to bind with Met, and their presence implies that DCP interacts with Met. DCP stimulates HCC cell proliferation through Met. Met is a membrane-spanning receptor tyrosine kinase that mediates biological responses to various tissues including cell scattering, growth

stimulation, and branching morphogenesis of cells in various tissues. ⁽⁷⁵⁾ DCP is a prothrombin precursor with no coagulation activity. The prothrombin precursor has 10 Glu residues that are converted into γ -carboxy-glutamic acid residues by glutamyl carboxylase. All of these Glu residues must be converted into glutamic acid residues before prothrombin can obtain coagulation activity. In DCP, not all of the 10 glutamic acid residues are transformed. Instead, some remain as Glu residues. ⁽⁷⁶⁾ Liebman et al. firstly reported the increasing levels of DCP in patients with HCC. Numerous studies indicated that DCP would be a useful marker in detecting HCC. ⁽⁷⁷⁾ DCP is a well-recognized tumor marker for its high sensitivity and specificity in the screening and diagnosis of HCC. ⁽³⁴⁾ Serum and tissue DCP expressions are thought to reflect the biological malignant potential of HCC. Serum DCP level is used as a clinical parameter for the development of portal venous invasion of HCC. ⁽⁷⁸⁾ DCP serum concentration is elevated in HCC patients compared with that in healthy adults and patients with nonmalignant hepatopathy. 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. ⁽³⁴⁾ Besides the purpose of screening HCC, serum DCP could also be used as a clinic pathological or prognostic indicator for HCC patients, and may be more useful than AFP in reflecting the invasive characteristics of HCC. ⁽⁷⁸⁾ Cui et al. 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. ⁽⁷⁹⁾ In a study by Marrero et al. AFP was found to be more sensitive than DCP and AFP-L3% for the diagnosis of early stage HCC at a new cutoff of 10.9 ng/ml. ⁽³⁴⁾ Other studies have found that DCP has a higher sensitivity for detecting and predicting the development of HCC. ⁽⁸⁰⁾ In a study of HCC patients, the sensitivity and specificity of AFP collected at the time of HCC diagnosis was 61% and 81% at a cutoff of 20 ng/ml and 22% and 100% at a cutoff of 200 ng/ml. Levels was measured 1 year before

diagnosis of HCC, the sensitivity and specificity at the low cutoff was 43% and 94%, respectively, for DCP and 47% and 75%, respectively, for AFP. A combination of these markers (AFP, AFP-L3, DCP) only improves the accuracy for early detection of HCC; the combination of tests may increase sensitivity but the specificity for detecting early HCC declines. ⁽⁸¹⁾

Matrixmetalloproteinases (MMPs)

Matrixmetalloproteinases (MMPs) are a group of zinc-dependent endopeptidases. Tissue remodeling occurs in various physiological and pathological processes including carcinogenesis, tumor progression and wound healing. ⁽⁸²⁾ Kuo et al. found that the presence of HBeAg was accompanied by a high activity of MMP-2. ⁽⁸³⁾ The relationship between hepatitis virus markers and MMPs was not elucidated in that study, HBeAg positive cases showed a tendency of high potential of portal vein invasion with strong expressions of MMP-7 and MMP-9. So, these MMPs cooperate mutually in the carcinogenesis, proliferation and infiltration of HCC through a close network. ⁽⁸⁴⁾ Significantly higher MMP-9/ MMP-2 ratios were found in advanced, inoperable HCC patients, compared to those in early stage HCC patients, so this ratio is useful in distinguishing between patients with early stage HCC and those with advanced HCC. ⁽⁸⁵⁾ Kim et al. reported that mRNA for MMP-14, MMP-15, and MMP-2 were expressed at high levels in most of the HCC cell lines. These MMPs play an important role in the growth, migration, invasion, and metastasis of HCC cells. Inhibitors selective for these MMPs would be more valuable reagents in preventing growth and metastasis of HCC. ⁽⁴⁷⁾ Liotta and Kohn, stated that MMPs play a central role in stromal invasion and subsequent metastasis in view of their ability to degrade many ECM components, stromal remodeling and release of growth factors from ECM. Gelatinases (MMP-2 and MMP-9) are the most important as they degrade type IV collagen which is one of the main component of bone marrow. ⁽⁸⁶⁾

Glutaminesynthetase (GS)

Glutamine synthetase catalyzes the synthesis of glutamine from glutamate and ammonia and plays an important role in

ammonia detoxification and nitrogen balance. ⁽⁸⁷⁾ Haupt et al. demonstrated the up-regulations of GS mRNA, protein and activity in human HCC. ⁽⁸⁸⁾ Also, Osada et al. reported stepwise increases in GS immune reactivity between precancerous lesions and early HCC, and from early HCC to advanced HCC, and proposed that GS play a role in the promotion of the metastatic potential of HCC. ⁽⁸⁹⁾ Glutamine is end product of GS activity, and is the main energy source of tumor cells. ⁽⁸⁾ Saitoh and Araki, found that a low glutamine concentration increases the half-life of GS protein. ⁽⁹⁰⁾ It was demonstrated that GS mRNA, GS protein, and GS activity are up regulated in human HCCs. GS was confirmed to be useful biomarker for early diagnosis and prognosis in HCC patients. ⁽⁹¹⁾ Also, they suggested that GS over expression is associated with tumor dedifferentiation.

Alpha L fucosidase (AFU)

AFU is a lysosomal enzyme responsible for hydrolyzing fucoseglycosidic linkages of glycoproteins and glycolipids. AFU is a glycosidase found in all mammalian cell lysosomes and is concerned with the degradation of a variety of fucose-containing fucoglyco-conjugates. ⁽⁹²⁾ The persistently elevated AFU level in sera of patients with liver cirrhosis contributes to early detection of primary hepatic carcinoma. ⁽⁹³⁾ Deugnier et al. found that raised serum AFU activity is more sensitive and specific than an elevated serum AFP concentration as a marker for HCC. The reason for the increase in serum activity of AFU in HCC is not known. The most likely explanation is that the raised serum concentrations result from an increased synthesis of proteins by the tumor with increased fucose turnover. ⁽⁹⁴⁾ The measurement of serum α -L-fucosidase activity levels may help in detection of liver carcinoma at an early stage, possibly improving the prognosis of patients with disease. Data support the clinical usefulness of measuring the serum α -L-fucosidase activity during the follow-up of cirrhotic patients and confirm that serum α -L-fucosidase activity may indeed be considered a reliable marker of HCC. ⁽⁹⁵⁾ AFU activity is not related to the tumor size. It has been proved that the values of AFU serum concentration were not correlated with the tumor size and were frequent in early HCC

cases. ⁽⁹⁶⁾ 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/h, and the activity of AFU is already elevated in 85% of patients at least 6 months before the detection of HCC by ultrasonography. ⁽⁹⁷⁾ According to study conducted on 884 Chinese subjects, the AFU activity was significantly higher in HCC patients compared to patients with cirrhosis, chronic hepatitis, other malignant tumors, other diseases and healthy individuals. The sensitivity for AFU was 81.5% and the specificity was 85.4%. ⁽⁹⁸⁾

In summary, tumor markers are very useful not only in early diagnosis of HCC tumor markers but also for good prognosis. Therefore these markers not only help in the early detection of HCC but may also give a hand in selecting the suitable therapeutic approach and embody new strategies and therapeutic intrusion.

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