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REVIEW

Serum Tumor Markers for Early Diagnosis of Primary Hepatocellular Carcinoma

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Abstract: Primary hepatocellular carcinoma (HCC) is one of the most frequently occurring pernicious tumors in the world. It is typically very insidious in the early stages with no obvious symptoms. Its development and metastasis are very rapid. Upon diagnosis, most patients have already reached a local advanced stage or have established distant metastases. The treatment of HCC is limited, with poor prognosis and short natural survival time. In order to improve the efficiency of early diagnosis, it is particularly significant to choose economic and effective diagnosis methods. Ultrasound, magnetic resonance imaging, and computed tomography are usually used in the clinic, but these methods are extremely limited in the diagnosis of HCC. Tumor markers have become the main effective early clinical diagnosis method. Potential serum tumor markers include alpha fetoprotein heterogeneity, Golgi protein 73, phosphatidylinositol proteoglycan (GPC-3), osteopontin, abnormal prothrombin, and heat shock protein. These tumor markers provide new ideas and methods for the diagnosis of HCC. A combination of multiple markers can make up for the deficiency of single marker detection and provide a new strategy for the prognosis and auxiliary diagnosis of HCC. This review introduces protein tumor markers utilized over the past five years.

Keywords: primary hepatic carcinoma, serum tumor marker, α-fetoprotein, alpha fetoprotein, heterogeneous Golgi protein 73, phosphatidylinositol proteoglycan, abnormal prothrombin, heat shock protein

Introduction

Hepatic carcinoma incidence and mortality rank fifth and second in all the tumor, respectively, and both of them have been gradually increasing. About 750,000 new cases of hepatic carcinoma are reported every year worldwide. Hepatic cellular carcinoma (HCC) is the most common pathological type of HCC, its prevalence reaching 90%.[1] HCC is characterized by aggressive invasion, rapid progress, and poor prognosis, which have seriously harmed people's health. Patients lack specific symptoms and signs in the early stages of the disease. Once diagnosed, they have already reached the middle and late stages. Therefore, it is of great significance to find a diagnosis method with a high accuracy to decrease disease mortality and increase patient survival time. Biomarkers are a type of markers related to cell growth and proliferation, as discovered in recent years with the development of immunology and molecular biology technology. They can be used to explore the pathogenesis on a molecular level and have unique advantages in accurate and sensitive evaluation of early and low-level damage. They can also provide early signals and evidence for auxiliary diagnosis for clinicians. Biomarker sensitivity can reach 82%, while specificity can reach 67%.[2] In addition, they are easily

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accessible, noninvasive, and low cost and therefore can be widely used in the clinic. Tumor cells produce and release certain substances, which often exist in tumor cells or host body fluids in the form of antigens, enzymes, hormones, and other metabolites. According to their biochemical or immune characteristics, substances with qualitative or quantitative changes in the body fluids, excluding substances and tissues from tumor patients, are called tumor biomarkers. There are three types of tumor biomarkers according to their function: 1. tumor biomarkers that can be used to distinguish different types of tumors; 2. tumor markers that can be used to predict and detect tumor occurrence, development, and metastasis; 3. tumor biomarkers that can be used to evaluate the effectiveness of treatment. The ideal biomarker should be highly specific, sensitive, and predictive. Serum tumor markers are indispensable and can be used to detect, diagnose, and judge the prognosis of HCC. By detecting common laboratory serum markers, early diagnosis and evaluation of pathological changes can be made and prognosis can be determined. Hepatic carcinoma biomarkers can be divided into four categories: embryo and glycoprotein antigens, enzymes and isoenzymes, genes, and cell factors.[3] With the continuous progress of biochemical technology in the 21st century, rapid progress has been made in the fields of gene technology, proteomics, and tumor immunity. A series of potential biomarkers have also been found. These biomarkers do not only contribute to the early diagnosis of hepatic carcinoma, but also help to comprehend its mechanism. They also provide more effective potential strategies for the treatment for HCC. This review discusses serum tumor markers for early diagnosis of HCC.

Alpha Fetoprotein (AFP)

Alpha fetoprotein (AFP) is one of the earliest protein tumor markers ever discovered. AFP comes from the endodermal organization cells in the embryo and has a molecular weight of 69,000 Dalton.[4] AFP accounts for 1/3 of the total plasma protein during the 13th week of fetal development and reaches its peak during the 30th week of pregnancy, decreasing gradually thereafter. AFP and other serum protein families are expressed in the blood. Different from other proteins, AFP loses almost all of its expression and can hardly be detected (<10 ng/mL) a few weeks after birth, concentration reaching about 1% of the peak. The concentration approaches adult levels (<30 μg/L) at the age of 1 year.[5] In 1964, Tatarinov et al found high concentrations of AFP in patients with hepatic carcinoma and in latter experiments confirmed that AFP is a sensitive index for diagnosis, efficacy evaluation, and prognosis of HCC.[6] Consequently, AFP has been used in the clinic ever since.

AFP is a 70-kDa carcinoembryonic glycoprotein composed of 591 amino acids and is a member of the serum protein family. It contains a carbohydrate chain that connects via a single aspartic acid. Its coding gene is situated on the long arm of chromosome 4. AFP-l1 (LCA non-responsiveness), AFP-l2 (intermediate reaction), and AFP-L3 (LCA affinity) are the three main types of AFP glycochain isomers. They have different affinity with lentil lectin and their expression levels are different under different physiological or pathological conditions.[7] The main function of AFP is to guarantee the transfer of various molecules, such as heavy metals, fatty acids, bilirubin, and some drugs in the embryonic and perinatal period. In addition, AFP has an immunosuppressive effect, which can prevent rejection of the fetus by the mother. The study shows that AFP is closely associated with malignant growth, metastasis, and tumor cell invasion.[8] It is believed that AFP is a key cell factor in drug resistance of hepatoma cells. It has potential biological properties of anti-apoptosis induction and can inhibit the biological function of PTEN and then cause hepatic cell apoptosis, which is induced by trans retinoic acid resistance. AFP can specifically bind to caspase-3, inhibit its activity, block the caspase signaling reaction cascade, and lead to the tolerance of hepatocarcinoma cells to TNF-related apoptosis-inducing ligand.[9] AFP has been used to screen and diagnose hepatic carcinoma since the 1970s. The positive rate of AFP in patients with HCC can reach 70–90%. Its specificity is as high as 72–90%, but its sensitivity is only 39–65%, and the effective rate of early diagnosis is only 9–32%.[10] About 1/3 of the HCC patients show negative AFP detection, especially for diagnosing early small HCC. About 30–40% of HCC, especially cholangiocarcinoma, have negative AFP expression.

AFP plasma concentration of 20 ng/mL is generally considered to be the pathological threshold in human beings, with a reference range of 200–300 ng/mL. It is generally believed that patients with serum concentrations >400 ng/mL can be diagnosed with hepatic carcinoma.[11] In HCC patients, AFP plasma level has a positive correlation with hepatitis B virus (HBV) infection, large tumor size, poor cell differentiation, and vascular invasion. AFP concentration can be affected by some diseases, such as acute or chronic hepatitis, liver cirrhosis, some gynecological tumors, and normal pregnancy.[12] AFP has gone through five stages of biomarker

development, but its routine application as a part of early detection and monitoring strategy for HCC is still controversial. High false negative rates (30% in advanced HCC and 40% in early stage HCC).[13] Therefore, extra biomarkers are needed to supplement AFP in order to improve the accuracy of HCC diagnosis. Joint detection of multiple markers can make up for the deficiency of AFP detection.

Alpha Fetoprotein Heterogeneity

In order to improve the diagnostic function of AFP, its three diverse variants (AFP-L1, AFP-L2, and AFP-L3) have been studied. AFP-L3 is a trehalose variant of AFP, which is unique to HCC. The results have shown that AFP-L3 expression makes up >10–15% of the total AFP and the specificity of HCC diagnosis is >95%.[14] AFP-L3 sensitivity can reach 80–90% when the tumor is >5 cm and only 35–45% when the tumor is <2 cm. Some studies have shown that AFP-L3 sensitivity is 60.25% and specificity is 78.37% in cirrhosis.[15] In HCC, the sensitivity and specificity were 67.74% and 81.52%, respectively. However, when the AFP concentration is <20 ng/mL, AFP-L3 cannot be detected. Therefore, the total concentration of AFP will affect AFP-L3 sensitivity. Although AFP-L3 specificity is very high, its low sensitivity limits its potential as a biomarker of HCC. At present, AFP-L3 is still recommended as a biomarker for early detection of HCC.[16] While, AFP-L3 is concern with histological grade of HCC.[17] Some studies have demonstrated that AFP-L3 sensitivity can be improved by using novel and advanced microfluidic separation technology.[18] HS-AFP-L3 can significantly improve the sensitivity and specificity of traditional AFP-L3. All of these indicate that HS-AFP-3 can be considered a valuable biomarker for the diagnosis of early HCC and can be used in clinical applications in the near future.

GOLPH 73 (GP 73, Also Known as GOLPH 2)

In 2000, Kladney et al first discovered Golgi protein 73 (GP73) when they studied the pathogen of adult giant cell hepatitis (GCH). GP73 is a transmembrane glycoprotein that exists on the cell membrane of Golgi cell in the tissue. The gene encoding GP73 is located in the 9q21.33 position of the short arm of chromosome 9 and its length is 3042 bp. It consists of a 1200-bp open reading frame containing two regions encoding 400 and 391 amino acids, respectively.[19] GP73 is expressed in many tissues in a normal human body, but hardly ever in hepatocytes. It can be detected only in a small amount of hepatocyte cytoplasm around the portal area. GP73 is expressed in the cytoplasm adjacent to the bile duct surface, but not adjacent to the membrane sinus surface. It is speculated that this protein has the function of a housekeeping gene. However, different expression levels of its mRNA and protein in different tissues suggest that the protein may have an effective regulatory ability and a variety of biological functions.[20] GP73 usually has a low concentration in the serum. However, when normal hepatoma cells are injured or infected, such as in viral hepatitis, alcoholic liver disease, autoimmune hepatitis, decompensated cirrhosis, and HCC, GP73 can circulate from the Golgi body smooth capsule, reach the intracellular body,[21] and release into the blood from the surface of the hepatoma cells. Levels of GP73 is related with HBV, HCV infections and IFN-γ; and GP73 could be regulated by IL-6, IL-1 and TGF-β.[20]

In 2005, Ai et al found that the level of GP73 in the serum of patients with HCC increased significantly. The earliest research on GP73 in China came from Mao Yilei in liver surgery at Peking Union Medical College Hospital in 2008. His team tested and compared AFP, GP73, and related HCC serum indexes of 37 patients with hepatitis B and HCC, 25 patients with hepatitis B virus, 12 patients without liver diseases, and 99 healthy volunteers.[22] The results showed that GP73 was detected in the sera of all patients. GP73 sensitivity was 76.9% and specificity was 92.8%. Mao et al took the lead in completing more than 4000 large-sample, multi-center, and multi-ethnic studies on the GP73 series in the world. Their results showed that GP73 has a specificity and sensitivity of 97.4% and 74.6% when diagnosing HCC, respectively.[23] Using large-sample data, GP73 has been demonstrated to be an ideal serum marker in the early diagnosis of HCC and assessment of postoperative recurrence. Its specificity and sensitivity are both superior compared to AFP. In gallbladder, lung, prostate, and other cancers, GP73 is highly expressed and is closely related to prognosis.[24] This indicates that GP73 has a potential clinical value for the diagnosis of multiple tumors.

GP73 expression was not affected by age, sex, tumor numbers, and serum alpha fetoprotein, but was related to tumor size, vascular invasion, and tumor differentiation. Some studies have shown that GP73 selectively interacts with Epidermal growth factor receptor (EGFR) and helps EGFR and other receptor tyrosine kinases anchor on the

membrane of circulating cells and across the Golgi network, thus promoting metastasis of HCC.[19] GP73 enhances the expression of matrix metalloproteinase-13 through transcription mediated by cyclophosphamide-responsive element-binding protein in order to enhance cancer cell invasiveness.[25] GP73 gene silencing reduced the expression of N-cadherin, E-cadherin, and key epithelial mesenchymal transition (EMT) factors, thereby reducing cell adhesion and promoting cancer cell movement. According to these studies, GP73 may be related to the progress and metastasis of HCC[26] and can thus be used as a new serum marker to monitor the recurrence of primary HCC, help clinicians determine treatment time, and improve patient prognosis and survival.[27] At present, GP73 is considered to be a diagnostic biomarker[28] and a probe for HCC-specific positron emission computed tomography to identify HCC in normal hepatic tissue and benign liver injury.

In order to further clarify the role of GP73 in tumor pathogenesis and to provide a new strategy for early tumor diagnosis, more extensive and in-depth research and clinical experiments are needed.[29] In addition, the clinical application of GP73 involves its use in predicting the prognosis of HCC after surgical treatment. Large-scale and multi-center investigations are still needed.[30]

Phosphatidylinositol Proteoglycan (GPC-3)

Phosphatidylinositol proteoglycan (GPC-3) is a member of the heparin sulfate glycoprotein family. It is anchored on the extracellular membrane via a combination of C-terminal and glycosylphosphatidyl alcohol. In 1996, Yale University first discovered the GPC gene (also known as MXR-7 or OCI-5 gene) in a study on Simpson-Golabi-Behmel syndrome, which is an X-linked recessive genetic disease. The gene is located on human chromosome xq26.10[31] and has a total length of more than 900 kb.[32] It is one of the largest genes in the human genome, consisting of eight exons and seven introns.[33] Arginine dissociates from serine at 359 and the N-terminal enters the blood to become soluble GPC3 with a molecular weight of 40,000 Dalton.[33,34] Some studies have shown that GPC-3 plays an important role in growth factor signal transduction, proliferation regulation, and differentiation and migration of tumor cells by regulating the activities of several tyrosine kinases and the Wnt signaling pathway.[28]

In the last few years, many studies have shown that GPC-3 is highly expressed in placental hepatic tissue, hepatic carcinoma tissue, and most hepatic carcinoma cell lines, but not in normal hepatic, lung, and adult kidneys. This evidence suggests that GPC-3 can be used as a valuable tumor marker for hepatic carcinoma histology. It has also been reported that GPC-3 is highly expressed in HCC with a sensitivity of 76.9% and a specificity of 96.8%.[35] The positive rate of HCC patients with a diagnosis diameter of <3 cm can be as high as 76%, while the expression level in cirrhosis nodules is very low. Thus, GPC-3 can identify benign and malignant tumor lesions. GPC-3 is upregulated in some HCC tissues compared to normal hepatocytes or hepatocytes with benign liver disease[36] and is thus considered to be a supplementary serological biomarker for AFP. GPC-3 can accurately distinguish small hepatic carcinoma, highly differentiated hepatic carcinoma, and potential liver cirrhosis and therefore has better diagnostic performance.[37] It has been speculated that the high expression level of GPC-3 in HCC patients may be due to the repeated expression of GPC-3 in embryos due to malignant pathological changes in hepatocytes or the interaction between upregulated growth factors and their receptors in HCC, leading to malignant development of hepatocytes.

Although many studies have shown that GPC-3 can be used as a specific biomarker for the diagnosis of hepatic carcinoma and a new target for its treatment, GPC-3's specific mechanism of action for the occurrence, development, and migration of hepatic carcinoma remains to be further verified. More accurate detection technology is needed to determine its expression in hepatic carcinoma cells,[38] as well as to improve the level of diagnosis with other biomarkers.

Abnormal Prothrombin (APT)

Abnormal prothrombin (APT) has a high positive rate in the serum of HCC patients and can reflect the tumor growth well.[39] It is considered to be an HCC marker with a high diagnostic rate and has been used as a serum tumor marker of HCC in Japan, Europe, and the United States for many years. APT is also known as des-γ-carboxy prothrombin (DCP)[40] and is an abnormal form of prothrombin, which can be found in the serum of patients with vitamin K deficiency or HCC. In the absence of vitamin K, hepatocytes cannot synthesize normal vitamin K-dependent coagulation factors (II, VII, IX, and X) and can only synthesize protein induced by vitamin

K absence or antagonist-II (PIVKA-II) without coagulation function.[41] Thus, it is a marker reflecting HCC.[42] APT is a serum biomarker approved and listed by the Food and Drug Administration (FDA) in the United States.

Abnormal serum prothrombin was initially detected by Liebman et al in 1984 in the sera of patients with hepatic carcinoma.[43] Prothrombin is synthesized by the liver when vitamin K is sufficient.[44] It has four functional domains: one γ-carboxyglutamic acid region (GLA region), two ring regions (kringle region), and one catalytic region. Among them, there are 10 vitamin K-dependent GLAs in the GLA region (mainly fragment aa1-40), which are located at the amino end of the peptide chain and are produced by the microsomal carboxylase system of hepatocytes. When vitamin K is sufficient,[45] these ten GLAs will all be converted into γ-carboxyglutamic acid, thus becoming normal prothrombin. When the vitamin levels in vivo are insufficient, vitamin K antagonist is present or cannot be utilized or one or more glutamate residues in the molecular structure are not fully carboxylated into γ-carboxyglutamic acid, thus losing their ability to combine with calcium ions and phospholipids as well as their clotting activity. Studies have confirmed that hepatic carcinoma tissue can produce PIVKA-II and release it into the blood for various reasons, but the mechanism of PIVKA-II production in hepatic carcinoma patients is not completely clear at present. It has been reported that the mechanism of PIVKA-II may vary: vitamin K deficiency is secondary to the intracellular transport mechanism of inadequate intake or dysfunction;[46] selective γ-carboxylase defect (to prevent the production of normal prothrombin); and when malignant transformation occurs in hepatocytes, the change in cytoskeleton damages the intake of vitamin K.

PIVKA-II and AFP are produced in different ways and their serum contents are relatively independent. Therefore, the determination of PIVKA-II and AFP is helpful for the diagnosis of hepatic carcinoma.[47] Joint detection in particular can improve the sensitivity of hepatic carcinoma detection, thus providing a complementary effect with AFP for the diagnosis of hepatic carcinoma. The study showed that the APT level in patients with hepatic carcinoma is significantly higher than that in normal people and there is no significant difference between patients with liver cirrhosis, chronic hepatitis, and some other malignant tumors.[48] There is also no significant difference among patients with chronic hepatitis, some other malignant tumors, and normal people, indicating that APT determination is of great significance for the differentiation of hepatic carcinoma and other benign liver diseases.

According to related worldwide reports, serum APT level is significantly related to tumor size, TNM stage, Child-Pugh score, relevant pathological characteristics, and tumor recurrence; A large body of clinical data shows that APT level is linearly related to the average hepatic carcinoma volume and tumor number, negatively related to the average survival time, and strongly related to the degree of HCC malignancy.[49] After tumor resection, a decrease in APT below the critical value may indicate that the operation was effective. However, if the level of serum APT rises again, it may indicate the recurrence or distant HCC metastasis. Dynamic APT monitoring can help to evaluate the occurrence, development, invasion, metastasis, or recurrence of hepatic carcinoma. APT has become an important index to evaluate patient prognosis. APT is especially helpful in making up for the deficiency of AFP in finding AFP-negative HCC and excluding AFP-positive non-HCC. Therefore, APT may be superior to AFP for hepatic carcinoma monitoring, early diagnosis, treatment response, and recurrence monitoring. The combination of APT and AFP will provide a more reliable basis for monitoring, treatment, and follow-up monitoring of hepatic carcinoma.

Heat Shock Protein (HSP)

Heat shock protein (HSP) was first found in the salivary gland of chromosome larva of heat shock Drosophila and then in prokaryotic and eukaryotic cells.[50] It plays the role of molecular chaperone under stress (including canceration). Although Heat shock proteins are indexes parameters obesity and oxidative stress, there has not been reports about NAFLD/NASH and type 2 diabetes related HCC. HSP70 is a type of a monomer protein that exists in the inner chamber of any eukaryotic cell containing adenosine triphosphate (ATP) and in the cell membrane.[51]

Immunohistochemistry results have shown that HSP70 is significantly expressed in early HCC compared to precancerous lesions, which may be due to inadequate blood supply or stimulation of hypoxic environment during the initial formation of hepatic carcinoma, resulting in an increase of HSP70 production and significantly high expression in advanced HCC.[52] Therefore, the most important clinical significance of HSP lies in the fact that it can be used as a sensitive indicator for the differential diagnosis of highly differentiated small hepatic carcinoma (SHCC) and highly differentiated atypical nodules. In the

context of malignant transformed cells, HSP promotes cell growth; inhibits aging; confers stress-resistant apoptosis; participates in cell inhibitory drugs and radiotherapy. HSP70 has the ability to stabilize the lysosomal membrane and affect autophagy,[53] thus promoting cancer cell survival. HSP70 plays an important role in the process of cellular loss of contact with the extracellular matrix during cancer metastasis. It has been reported that HSP70-14 is upregulated in the tumor tissue of HBV related to early HCC.

Hsp90α is a conserved and necessary molecular chaperone involved in many physiological and pathological signaling pathways, which can be transported to the cell surface by cancer cells and secreted into the extracellular space. Plasma HSP90α has significant sensitivity and specificity for the detection of hepatic carcinoma patients, especially in patients with early or small hepatic carcinoma (<3 cm), nonhepatic carcinoma patients, and high risk controls. HSP90 AA1 expression in HCC is significantly higher than that in normal liver tissue, which can be used as a potential biomarker for diagnosis and prognosis of HCC. HSP90AA1 is also highly expressed in most other cancer types. Although the extracellular effect of HSP90 AA1 is not completely clear, it is known to promote metastasis. As a biomarker for clinical diagnosis of HCC, plasma HSP90α is expected to become a good disease marker for three reasons: 1. It is highly expressed in hepatic carcinoma cells, accounting for 2–7% of the total protein; 2. It can be transported to the cell surface or secreted by different types of hepatic carcinoma cells; 3. The change in HSP90 expression is related to tumor occurrence, progression, and metastasis. Whether the level of HSP90α in the plasma of patients with HCC can improve the accuracy of diagnosis and predict the response to treatment still needs further large-scale research.

HSPA5 stress induction plays an important role in the occurrence, metastasis, and treatment of drug resistance in HCC.[54] Its clinical efficacy as a biomarker of diagnosis and prognosis of HCC deserves further clinical verification.

Dickkopf-1 (DKK1)

DKK1 is a secretory glycoprotein. Human DKK1 gene is situated at chromosome 10q11.2 and encodes 266 amino acid proteins. It is an excretion antagonist of the Wnt/β-catenin signaling pathway. It inhibits the activation of a typical Wnt pathway and induces lipoprotein receptor-related protein (LRP) endocytosis through competitive binding receptor LRP (low-density lipoprotein receptor)-5 and −6 and Kremen protein,[55] thus preventing Wnt-frizzled-LRP5/6 receptor recombination. The formation of β-catenin enables it to enter the nucleus and interact with members of the T-cell factor (TCF) family. TCF family regulates the Wnt target gene, promotes the mutation of Wnt signaling pathway structural activation, and finally leads to the occurrence of cancer. Its exact function is not completely clear. It has been reported that in chronic inflammatory diseases such as various types of cancer, rheumatoid arthritis, and lupus, the concentration of DDK1 in the peripheral blood is increased. It also has a high specific expression in cancer cells, including multiple myeloma, prostate cancer, and hepatic carcinoma. DKK1 may be a tumor-specific serum biomarker for human cancer.[56]

In a cohort study, the values of DKK1 and AFP were measured in 1284 patients (831 in the experimental group and 453 in the control group). The results indicated that the concentration of DKK1 in hepatic carcinoma patients was significantly higher than that in all the control groups and there was no significant difference between the control groups ($P<0.001$).[57] The sensitivity of this test was 73.8% and specificity was 87.2%. The detection of DKK1 is more useful for the detection of AFP-negative HCC. The combination of AFP and DKK1 is slightly better than AFP alone for the early detection of HCC. In addition, there has been a large-scale multi-center Phase II study to detect serum DDK-1 values in patients with hepatic carcinoma, chronic HBV, and cirrhosis.[58] The results show that the increase in serum DKK1 concentration is helpful to identify the patients with liver cirrhosis and HBV infection (Area Under The Curve (AUC): 0.693 vs 0.691). Some studies have shown that the higher preoperative serum DKK1 level is related to the lower overall survival rate and the overall median survival time.[59]

DKK1 serological level can be used as a sensitive and noninvasive test for early diagnosis and prognosis of HCC[60] This testing method can make up for the deficiency of existing serological diagnosis, but further long-term follow-up study is needed to clarify the diagnostic and prognostic value of serum DKK1 in HCC patients. In addition, a large-scale prospective investigation is needed to estimate the sensitivity and specificity of DKK1 in HCC more accurately.

Osteopontin (OPN)

Osteopontin (OPN), also called transformation-associated protein phosphatase, is a phosphoprotein that integrates with integrin. It is also a chemokine, calcified multifunctional extracellular matrix[61] secreted at a low level by bile duct epithelial cells. Under normal circumstances, OPN is expressed in bile duct epithelium, stellate cells, and Kupffer cells, but not in hepatocytes. In addition, some studies have shown that OPN is overexpressed in lung cancer, breast cancer, colon cancer, and hepatic carcinoma.[62]

In 1979, OPN was originally described as a phosphoprotein that is secreted by transformed malignant epithelial cells.[63] OPN is mainly produced by the immune system and epithelial cells, although tumor cells, smooth muscle cells, and osteoblasts can also release OPN. OPN interacts with the integrins and CD44 receptor family, enhances hepatocyte growth factor (HGF)-induced scattering and invasion, and activates c-Met to promote the progression of hepatic carcinoma. OPN can also bind with vimentin and improve its stability by inhibiting protein degradation, thus improving the EMT in HCC cells. In addition, OPN is considered to be a chemoattractant of macrophages and neutrophils in inflammatory liver disease.[64] The level of plasma OPN in HCC patients was significantly higher than that in healthy individuals and chronic liver disease patients. It has been reported that OPN sensitivity can reach 87% and maximal specificity can reach 82%. In a Phase III validation study of 131 HCC and 76 cirrhosis patients, OPN was superior to AFP in early detection. In a meta-analysis, the sensitivity and specificity of OPN for all stages of HCC can reach 86%. In a cohort study containing 312 healthy adults, cirrhosis, chronic hepatitis, and hepatic carcinoma patients, OPN concentration was detected with a threshold of 91 ng/mL and its relative sensitivity to AFP was increased. The sensitivity and specificity were also increased when the threshold of OPN and AFP were at a level of 156 ng/mL and 20 ng/mL, respectively.[65] In addition, the sensitivity of OPN combined with AFP in detecting HCC was 82%, while specificity was 77%.

OPN is a promising diagnostic biomarker of HCC. When OPN is combined with AFP and other biomarkers, the detection rate of HCC increases, heightening both sensitivity and specificity. In addition, OPN is also used as a prognostic marker of HCC. However, large-scale studies are still needed to verify its significance in liver inflammation, fibrosis, and cancer-related cell interactions. The role of pathophysiology in molecular mechanisms helps to develop new strategies for early diagnosis, monitoring, and therapy of liver diseases in order to further confirm its potential therapeutic value for HCC.

Conclusion

Pathogenesis of tumor formation/development is related with multiple factors and genes and its occurrence and development processes are complex. Some tumors are difficult to locate in the early onset stages, making the diagnosis challenging. Even if a tumor can be clearly diagnosed, sometimes it lacks effective treatment measures, its natural survival time is short, and its prognosis is poor, which is a serious burden on patients, families, and society. Therefore, there is an urgent need for effective diagnosis and treatment. Tumor markers are an effective, noninvasive, and economical tumor diagnostic method. The ideal tumor markers should have the following characteristics: 1. high specificity, can only be detected in the tumor patients; 2. high sensitivity, can be detected when samples are small; 3. organ specificity; 4. closely related to tumor stage; 5. closely related to patient prognosis; and 6. good predictive effect. Such tumor markers can be used for diagnosis (screening project), prognosis, monitoring treatment effect, and as targets for location and treatment. In the present review, we summarize eight serum tumor markers for early diagnosis of HCC: AFP, Alpha fetoprotein heterogeneity, GOLPH 73, GPC-3, APT, HSP, DKK1, OPN, which seem no reports with Barcelona Clinic Liver Cancer staging (BCLC staging). However, a single biomarker has limited detection capability for specific organs, with poor specificity and sensitivity. At the same time, there is not an "ideal" tumor screening tool for biomarkers to meet all of the requirements for diagnosis, prognosis, and prediction at the same time.

Although some studies have reported serum tumor markers with potential value for hepatic carcinoma, many problems associated with the research on these tumor markers exist: 1. relevant research studies have small sample sizes, are single-center, lack large-scale and multi-center sample data support, and have not had effective follow-up for clinical verification; therefore, large sample sizes and multi-center data are needed to be performed (Table 1). 2. the experimental design is incomplete and there has been no clear research to distinguish hepatic carcinoma from benign lesions; 3. lack of strict detection threshold. The specific mechanism of these potential biomarkers in the occurrence, development, and migration of HCC remain unclear. In addition, with the development of precision medicine, it

Table 1 Research Types and Number of Samples of the Disease Markers

	Research Types	Number of Samples	Reference No.
AFP	Original article	836	Reference [10]
AFP-L3	Original article	110	Reference [17]
GOLPH 73	Original article	352	Reference [20]
GPC-3	Original article	30	Reference [20]
APT	Original article	215	Reference [42]
HSP	Original article	220	Reference [54]
DKK1	Original article	831	Reference [57]
OPN	Original article	131	Reference [65]

is necessary to combine large-scale clinical data with biomarker research and to develop a high-throughput, highly sensitive, highly repetitive, and sample-compatible detection platform through high-throughput sequencing and mass spectrometry-based expression spectrum analysis. With the fast development of nano-technology, there have been several types nano particles for diagnosis of HCC,[66] Thus, more research and experiments are needed to further objectively evaluate these potential swelling tumor markers.

Disclosure

The authors report no conflicts of interest for this work.

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