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REVIEW

Serum Markers of Hepatocellular Carcinoma

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Abstract
Background The hepatocellular carcinoma is one of the most common malignant tumors and carries a poor survival rate. The management of patients at risk for developing HCC remains intricate.
Methods A literature search identified potential markers for hepatocellular carcinoma. These markers were analysed and justification was provided for these factors' inclusion to (or exclusion from) the markers of hepatocellular carcinoma (HCC). A search of the literature was made using cancer literature and the PubMed database for the following keywords: "markers and HCC," "Lens culinaris agglutinin reactive AFP (AFP-L3) and HCC," "Des-γ-carboxy prothrombin (DCP) and HCC," "Glypican-3 and HCC," "Chromogranin A and HCC," "Transforming growth factor β1(TGF) and HCC," "α-l-fucosidase (AFU) and HCC," "Golgi protein-73 (GP73) and HCC," "Hepatocyte growth factor (HGF) and HCC," "Nervous growth factor (NGF) and HCC."
Conclusions Despite the large number of studies devoted to the immunohistochemistry of HCC, at the present time, the absolute positive and negative markers for HCC are still lacking, and even those characterized by very high sensitivity and specificity do not have an universal diagnostic usefulness. Given the poor response to current therapies, a better understanding of the molecular pathways active in this disease could potentially provide new targets for therapy. However, AFP shows a low sensitivity, therefore other biomarkers have been developed to make an early diagnosis and improve patients' prognosis.

Keywords Hepatocellular carcinoma · Alpha-fetoprotein (AFP) · Lens culinaris agglutinin reactive AFP (AFP-L3) · Des-γ-carboxy prothrombin (DCP) · Glypican-3 (GPC3) · Chromogranin-A(CgA) · Transforming growth factor β1 (TGF-β1) · Alfa-l-fucosidase (AFU) · Golgi protein 73 (GP73)

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and the third most common cause of cancer-related death [1]. The most important risk factors for HCC are chronic hepatitis B or C infection, cirrhosis, non-alcoholic fatty liver disease (NAFLD), alcohol-induced liver disease (ALD), and exposure to aflatoxin and other carcinogens [2–7]. The clinical manifestations of HCC include abdominal pain in the right hypochondrium, hepatomegaly, and weight loss. The diagnosis of HCC is usually based on the atypical histopathology combined with the laboratory screening including index of hepatic damage (alanine aminotransferase and aspartate aminotransferase), the index of cholestasis (alkaline phosphatase

and gamma-glutamyl transpeptidase), the index of hepatic synthesis (albumin, prothrombin time, bilirubin) and finally, tumor markers and instrumental tests, which include hepatic ultrasonography, computed tomography (CT), nuclear magnetic resonance (NMR), and angiography. The best therapy for HCC is surgical hepatic resection, but when this is not possible, other treatments may be utilized such as systemic chemotherapy, hepatic intra-arterial chemotherapy (HIAC), transcatheter arterial embolization and chemoembolization (TACE), percutaneous ethanol injection (PEI), and hormone treatment [8]. So far, alpha-fetoprotein is the most common marker used in clinical practice, in conjunction with hepatic ultrasonography, to detect HCC in cirrhosis patients. An early diagnosis of HCC is extremely important in improving the survival of patients. The identification of biological markers of HCC recurrence and metastasis is indispensable for the proper management of HCC.

In this review, we attempt to collect the wide-ranging body of existing literature on this subject. The motivation behind this effort is that each existing marker alone is poorly specific to predict this disease. Most markers are not related to each other. False-negative results may significantly contribute to an incorrect diagnosis and using more than one marker at a time should greatly reduce the chance of errors from false-negative results.

Markers

Alpha-Fetoprotein

Alpha-fetoprotein is a glycoprotein with a molecular weight of about 70 kDa. Under physiological conditions, AFP is synthesized by the embryonic liver cells of the vitelline sac and fetal intestinal tract in the first trimester of pregnancy.

The *AFP* gene is expressed in hepatocytes and endodermal cells of the yolk sac during fetal life. Its expression is reduced after birth. The elevation of AFP occurs in hepatocyte regeneration, hepatocarcinogenesis, and embryonic carcinomas.

The biological function of AFP is still not well identified. Since AFP is similar to albumin, it is possible that AFP function as a carrier for several ligands such as bilirubin, fatty acids, steroids, heavy metals, flavonoids, phytoestrogens, dioxin, and various drugs [9, 10]. The increase of AFP levels >500 ng/ml is correlated with the tumor size: 80% of small HCC show no increase of AFP concentration. Furthermore, sensitivity of AFP decreases from 52 to 25% when tumor diameter is >3 and <3 cm, respectively [11]. Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP without the presence of tumor. The clinical use of AFP has been indicated principally (1) to execute the screening and diagnosis of HCC in patients at risk of developing HCC. In this case the measurement of AFP level is accompanied by hepatic ultrasonography; (2) as a marker for detecting tumor progression in patients with AFP-producing HCC. After treatment of the tumor, complete response is likely if the pre-treatment-elevated AFP levels decline to normal levels during subsequent follow-up measurements; (3) In staging: one of the most important staging systems for HCC is the CLIP (Cancer of the liver Italian program) staging system. The CLIP system assigns a score to the following four independent factors:

- Child–Pugh's classes
- Tumor morphology
- AFP concentration: (higher or lower than 400 ng/ml)
- Portal vein thrombosis

The CLIP system was used to define the parameters of liver function and tumor characteristics to establish a prognosis for HCC patients, and patients are followed-up to monitor the response to treatment. The measurement of AFP serum concentration during the follow-up of patients after treatment is a helpful test in conjunction with computed tomography or magnetic resonance imaging [12]. A decrease of AFP levels less than 10 ng/ml within 30 days is sufficient to establish treatment effectiveness [13]. Reduction of AFP levels after palliative treatment, such as with transarterial chemoembolization, indicates a favorable response to treatment. However, the evaluation of serum AFP concentration is clinically significant when AFP is elevated before the therapy.

Lens Culinaris Agglutinin Reactive AFP

There are several AFP glycoforms that differ in the binding affinity to lectins such as Lens culinaris agglutinin (LCA). The AFP glycoforms include: AFP-L1 or LCA no reactive is the principal AFP isoform in patients' serum with chronic hepatitis and liver cirrhosis; AFP-L2 presented intermediate affinity to LCA. It is produced by yolk sac tumors and could also be detected in maternal serum during pregnancy; Lens culinaris agglutinin reactive AFP (AFP-L3%) or Lens culinaris agglutinin reactive fraction of AFP, has an elevated affinity to LCA. The latter isoform has 1–6 fucose residues attaching at reducing terminus of *N*-acetylglucosamine and is derived only by cancer cells, so it has been reported to be a more specific marker for HCC [14, 15]. AFP-L3% should be used as a supplemental test in those patients with elevated total AFP. However, the clinical utility of AFP-L3% and the ratio of AFP-L3% to total AFP remain unclear. AFP-L3% levels have been found to be related to progression from moderately

differentiated to poorly differentiated tumors [16]. The cut-off for AFP-L3% is set up >10% of total serum AFP. AFP-L3% measurement for HCC has a specificity >95% [17, 18] and a sensitivity of approximately 51%. Therefore, it may be used as an early diagnosis of HCC when the tumor diameter is <2 cm. The sensitivity of AFP-L3% changes with HCC along with clinical stages: in small HCC (diameter <2 cm) AFP-L3% shows a sensitivity of around 35–45%, while its sensitivity reaches 80–90% when the tumoral diameter is >5 cm [18]. Since AFP-L3%-positive patients develop early vascular invasion and intrahepatic metastasis, AFP-L3% is also considered as a marker for the aggressiveness of HCC. In this regard, it was suggested that AFP-L3% expression is connected with increased nuclear expression of Ki67 (an indicator of the aggressive nature of cancer) and with decreased expression of α-catenin, which is associated with distant metastasis [19].

Moreover, there is a relationship between AFP-L3% levels and histological grade [20, 21] underscored by evidences that AFP-L3%-positive patients show poorly differentiated tumor. AFP-L3% is used not only for prognostic information [22, 23] but also in the patients' follow-up after initial treatment [24]. In fact, it is an indicator of poor prognosis for HCC and of metastasis [25]. Moreover, patients positive for AFP-L3% after therapy show a shorter survival than those who are AFP-L3%-negative.

Des-γ-Carboxy Prothrombin

Des-γ-carboxy prothrombin (DCP) or prothrombin induced by vitamin K absence (PIVKA) is an abnormal prothrombin derived by an acquired defect in the post-translational carboxylation of the prothrombin precursor in HCC cells [26]. DCP derived by reduction γcarboxylase activity that resulted in a lack of γ-carboxylation of the glutamic-acid residues. The reduced activity of γ-carboxylase was attributed to defective gene expression in HCC patients [27]. There are various differences between DCP and total AFP. First of all, DCP is a more specific HCC marker than AFP because other liver diseases don't cause an increase of DCP serum levels. DCP measurement for HCC has a sensitivity of 48–62% and a specificity of 81–98% [28]. The accuracy of DCP is decreased in prolonged obstructive jaundice, intrahepatic cholestasis with vitamin k deficiency, and intake of warfarin. Furthermore, DCP serum half-life (of around 40–72 h) is shorter than AFP serum half-life (of around 5–7 days), so DCP allows valuing the therapeutic efficacy of HCC in a timelier manner. DCP measurement in HCC patients is connected with the prognosis. In fact DCP high levels are associated with a poorer prognosis [29]. Lastly, there is no correlation between DCP levels and total AFP levels.

DCP- positive patients frequently develop portal vein invasion, intrahepatic metastasis, hepatic vein thrombosis, and capsular infiltration [30]. Additionally, DCP is considered a clinical marker for the development of portal vein invasion which leads to intrahepatic metastasis [31, 32]. DCP is involved in tumoral angiogenesis: recent studies have shown that DCP is able to augment the proliferation and migration of human vascular endothelial cells [33] and there is a correlation between the cell proliferation marker as PCNA and DCP tissue expression in HCC [34]. In fact, not only does DCP function as a growth factor, it is also able to increase genic expression of angiogenic factors such as EGF-R, VEGF, and MMP-2.

Glypican-3

Glypican-3 (GPC3) is one of the members of heparan sulphate proteoglycans [35]. It binds to the cell membrane through the glycosil-phosphatidylinositol anchors. GPC3 interacts with several growth factors [36] and this interaction regulates positively or negatively (depending on the specific growth factors) the growth factor activity [37]. Usually, GPC3 has a role in regulating cell proliferation and survival during embryonic development by modulating the activity of various growth factors. It also acts as a tumor suppressor [38]. GPC3 is mutated in patients with Simpson-Golabi-Behmel syndrome, an X-linked disease [38].

Recent studies have shown that GPC3 levels are increased in HCC patients [39, 40]. GPC3 is able to differentiate between malignant and benign hepatic lesions [40]; in fact, GPC3 levels are undetectable in healthy subjects and in benign hepatic disease patients (such as dysplastic or cirrhotic nodules). When GPC3 is overexpressed, it acquires a new function that lacks in normal tissues [41, 42]. Since the heparin sulphate chains of GPC3 interacts with heparin-binding growth factors and other growth factors such as HGF and VEGF, can contribute to the development of hepatic cancer.

P-aPKC-ι, E-Cadherin, β-Catenin

P-aPKC-ι, E-cadherin, and β-catenin play an important role in tight-junctions formation among tumor cells. P-aPKC-ι is a member of the family of serine-threonine kinases (PKC) that play an important role in cellular proliferation and differentiation [43]. P-aPKC-ι is very important for apicobasal maintenance and cellular junction formation [44]. Recent studies have shown that atypical PKC-ι is highly expressed in some malignant tumors and its expression level is correlated to the genesis, development, and prognosis of cancer [45, 46]. The P-aPKC-ι expression is increased in HCC and is higher in

undifferentiated cancer than in well-differentiated cancer. In normal liver tissue, P-aPKC-ι is localized at the apical membrane, while in HCC tissues it is localized at the basal membrane and in cytoplasm [46]. The high expression of aPKc-ι caused the loss of cell polarity and cellular junction that lead to metastasis.

E-cadherin and β-catenin-mediating intercellular adhesion are involved in invasion and metastasis of the cancer [47]. E-cadherin is a transmembrane glycoprotein and its intracellular domain is connected, through β-catenin and other catenins, to the acting cytoskeleton. E-cadherin is more expressed in well-differentiated tumors than in poorly differentiated cancers that have lost intercellular adhesion and have developed metastasis [48]. E-cadherin is considered a marker of tumor differentiation [49]. The reduced expression of E-cadherin, through the inhibition of the formation of a tight junction among tumoral cells, is correlated to insufficient tumoral differentiation and development of metastasis.

Regarding β-catenin, its cytoplasmatic overexpression in HCC tissues is involved in activation of the WNT signaling pathway. Additionally, β-catenin induces the gene expression of c-myc, cyclin D, VEGF, and other genes that increase cell proliferation.

Human Carbonyl Reductase 2

Human carbonyl reductase 2 (HCR2) gene encodes a cytosolic enzyme that is expressed in the human liver and kidney. This enzyme is important in detoxification of the reactive α-dicarbonyl compounds and reactive oxygen species (ROS) deriving from oxidative stress. In HCC, the antioxidant defense system including HCR2 and glutathione-S transferase (GSH) is repressed. This altered detoxification system is involved in HCC progression [50]. Therefore, the decreased expression of HCR2 in HCC tissues contributes to cancer growth because it increases the cellular damage induced by ROS and other carcinogens. The HCR2 levels are inversely correlated to the pathological grading of HCC: lower HCR2 expression is detected in advanced lesions [51].

α-l-Fucosidase

α-l-fucosidase (AFU) is a lysosomal enzyme found in all mammalian cells and is linked to the degradation of a variety of fucose containing fuco glycoconjugates [52]. Its activity is higher in HCC patients than in healthy individuals and in chronic hepatic disease patients. The cut-off value is set to 870 nmol/l. AFU shows a sensitivity of 81.7% and a specificity of 70.7%. There is no correlation between AFU serum concentration and AFP levels or alanine aminotransferase (ALT) activity. So, the increased AFU levels in HCC patients is not related to liver regeneration or necrosis but probably associated with an increased synthesis of protein that leads to an increase in fucose turnover [53]. Nevertheless, this explanation is not supported by recent studies that show a decrease of AFU expression in tumoral liver tissues compared to normal tissues [54]. AFU measurement is useful in association with AFP in the early diagnosis of HCC [55]. Moreover, there is a positive correlation between AFU levels and tumor size in HCC patients [56]. The AFU increase has been observed in non-cancerous extrahepatic disease such as diabetes, pancreatitis, and hypothyroidism.

Vascular Endothelial Growth Factor

The development of solid tumors is strictly correlated with angiogenesis. Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis: it stimulates the proliferation and migration of endothelial cells and increases vascular permeability. VEGF is highly expressed in various human cancers [57–59]. HCC shows an elevated expression of VEGF [60, 61], and particularly increased VEGF expression is present in advanced HCC compared to early HCC. Moreover, VEGF levels are higher in HCC patients than in chronic hepatic disease patients. VEGF is produced by HCC cells but the plasma VEGF elevation in advanced HCC suggests that other mechanisms are involved in the increase of VEGF levels. Vascular damage and invasion by cancer cells are fundamental for distant metastasis. Vascular injury causes the agglutination and platelet activation. Platelets, activated by vascular invasion of HCC cells, release VEGF [62]. As consequence, the increased vascular permeability induced by VEGF makes easier the VEGF passage into circulation. Therefore, VEGF is considered a possible tumor marker for the metastasis of HCC. High serum VEGF is associated with portal vein emboli, poorly encapsulated tumors, microscopic vein invasion, and recurrence in HCC patients [63]. VEGF is a predictor of tumor aggressiveness, disease-free survival, and overall survival in patients who underwent HCC resection.

Squamous Cell Carcinoma Antigen (SCCA)

SCCA belongs to the high-molecular-weight family of serin protease inhibitors (serpins) [64]. There are two different isoforms that are expressed in the suprabasal layer of multi-stratified squamous epithelium [65]. SCCA expression, as well as AFP production, could be the consequence of the dedifferentiation often observed in HCC. Since there is an important difference between SCCA expression in

HCC and in peritumoral tissues in the same patients, it can be used in immunohistochemical diagnosis of HCC or to explore micrometastasis [66]. HCC patients show higher SCCA serum levels than cirrhotic patients [66]. There is no clear correlation between SCCA expression tissue and SCCA serological levels because SCCA is expressed in the cytosol and is not associated with the cellular membrane. Conceivably, circulating SCCA is not secreted by cells, but derived by cellular lysis [67]. SCCA may be used for HCC diagnosis as it shows a sensitivity of 84.2% and a specificity of 48.9%. Given that SCCA is inversely correlated with tumor size, it is helpful for early HCC diagnosis and in screening of chronic hepatic disease patients.

Chromogranin A

Chromogranin A (CgA) is an acidic glycoprotein contained in secretory granules of neuroendocrine cells [68]. Many studies show high serum Cg-A concentration in patients with HCC, suggesting that CgA might represent a useful marker for HCC [69]. CgA levels are increased in other tumors such as pancreatic and prostate cancer [70, 71]. Spadaro et al. [72] report that the determination of CgA serum values is useful in monitoring cirrhosis patients for the early detection of an increase or decrease of HCC Cg-A levels according to the degree of neuroendocrine differentiation of HCC.

Moreover, CgA degradation is decreased because of progressive hepatocellular failure. The correlation between circulating CgA levels and histological stage of fibrosis suggests that CgA may be involved in hepatic fibrogenesis. Since CgA levels increase in both HCC patients and in cirrhotic patients, it shows a low diagnostic specificity. However, CgA concentration is a useful indicator for assessing neuroendocrine differentiation in connection with the stage of HCC. Patients with a higher CgA serum concentration show a poorer outcome than those with lower CgA levels [73]. Moreover, CgA serum concentration is increased in patients with neuroendocrine tumors that have metastasized to the liver [74]. In these patients, a positive correlation between the tumor size and CgA serum levels has been reported [75]. In contrast, CgA serum concentration is rarely increased in patients with small neuroendocrine tumors. Additionally, CgA can be utilized in HCC treatment.

Transforming Growth Factor β1

Transforming growth factor β1 (TGF-β1) is a negative factor in tumor growth: it arrests the cell cycle in the G1 phase, inducing inhibition of cell proliferation and triggering apoptosis [76]. In normal liver tissues, TGF-β1 is produced only by nonparenchymal cells (Kupffer cells, storing cells, and endothelial cells). Many studies report an up-regulated expression of hepatic TGF-β1 in tumor cells, including HCC. Recent studies show that TGF-β1 serum levels are increased in HCC patients [77]. TGF-β1 is secreted by HCC cells and there is an over-expression of the TGF-β1 gene in HCC cells [78]. The increased expression of TGF-β1 in HCC is correlated with hepatocarcinogenesis, since it not only inhibits the recognition of tumor by immunological system and the immune-mediated cytolysis but also promotes tumor angiogenesis [79, 80]. The expression of TGF-β1 mRNA tends to be higher in the patients with increased AFP and ALT levels while decreased TGF-β1 mRNA expression is correlated with the change of platelets count. The levels of TGF-β1 mRNA are higher in patients with advancing histological aggressiveness: in general, in the larger tumor the TGF-β1 mRNA expression is higher. It is important to mention that TGF-β1 induces growth inhibition in epithelial cells through a reduction of cyclin D expression in several tissues [81]. HCC cells show resistance to TGF-β1 growth inhibition because in tumoral cells there is an overexpression of cyclin D1 correlated with the dysregulation of the cell cycle and tumor progression [82, 83].

Golgi protein-73

Golgi protein-73 (GP73) is a resident Golgi glycoprotein expressed in epithelial human cells [84]. Physiologically, GP73 is expressed in biliary epithelial cells but not in hepatocytes. In liver disease, GP73 expression is increased in hepatic cells [85]. Moreover, Gp73 serum levels are increased in chronic liver disease patients, particularly, GP73 values are higher in early HCC patients than in cirrhotic patients [86]. GP73 is considered a possible marker for HCC, in fact it shows a specificity of 75% and a sensitivity of 69%.

Since GP73 is a Golgi-resident protein, its presence in circulation is surprising. A possible explanation of the detection of GP73 serum can be that this protein is able to arrive to the plasma membrane and pass into the circulation. There are several isoforms of GP73 correlated with different levels of glycosylation [87]. Therefore, some isoforms are more specific for HCC. Further studies are needed to confirm the role of GP73 in HCC diagnosis.

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF) is a cytokine having a wide range of effects, from embryonic development and liver regeneration to protection and/or repair of various organs, including kidney, lung, and cardiovascular system [88, 89]. The principal and most successful therapy for HCC is hepatic resection when the patient maintains good

liver function [90]. The pre-operative evaluation of hepatic function is very important to avoid liver failure [91]. Since good liver function prolongs the survival of patients that can receive further therapies, the liver function examination is very useful in predicting post-operative complications and survival after surgery. HGF stimulates hepatocyte proliferation including HCC cells [92] through expression of its receptor, the c-met receptor.

Hepatocyte growth factor (HGF) is detected in the serum of hepatic chronic disease patients. There is a correlation between HGF serum values and a worsening of liver disease [92].

The increase of HGF serum levels in cirrhotic patients is an indicator of HCC development [93]. HGF serum levels higher than or equal to 1.0 ng/ml have been correlated with poor survival. Therefore, pre-operative high HGF levels are related to development of post-operative complications, such as liver failure [94] and a poor survival. HGF can be helpful in assessing hepatic function before surgery and for predicting a patient's prognosis. Moreover, elevated HGF serum levels, after surgery, is able to predict early tumor recurrence and metastasis [95].

Serum Anti-p53

The p53 gene is an onco-suppressor gene encoding a nuclear phosphoprotein (p53 protein) that inhibits cellular proliferation and transformation [96]. Mutations of the p53 gene have been reported in several human cancers. P53 alterations occur at the late stages of hepatocarcinogenesis. Therefore, p53 alteration is not an early event in HCC and it is connected with the prognosis and survival of HCC patients. Mutated p53 proteins for its prolonged half-life are liable to accumulate in tumoral cells [97]. In fact, there is a correlation between p53 gene mutations and protein accumulated [98]. This correlation makes possible the use of simple immunologic methods for p53 detection. P53 mutations are correlated with poorly differentiated cancer and shorter survival of patients with HCC [99]. Mutant p53 proteins can be released in the serum by tumor cells; therefore, antibodies to p53 protein have been detected in HCC and in other tumors such as breast cancer [100], lung cancer [101], prostate cancer, leukemia, B-cell lymphoma, thyroid cancer, and pancreas cancer [102]. P53 alterations are detected in 30–50% of HCC patients [103] and these abnormalities are associated with a poor prognosis of HCC patients.

Nervous Growth Factor

Nervous growth factor (NGF) is involved in aspects of tumor biology such as growth invasion and metastasis, in addition to its role in differentiation and survival of neuronal cells. NGF can interact with two types of cell membrane receptors: TrkA NGF and p75NGF [104]. TrkA is a high-affinity receptor with tyrosine kinase activity and binding results in intracellular signaling through the mitogen-activated protein kinase and phosphatidylinositol-3-kinase cascades [105]. p75NGF is a low-affinity glycoprotein receptor. p75NGF structurally resembles members of the p55 tumor necrosis factor receptor family and has no tyrosine kinase activity and binding of NGF stimulates recruitment of cytoplasmic factors to the intracellular domain of the receptor that may lead to either apoptosis or cell survival [106, 107]. Various studies show that NGF is over-expressed in approximately 60% of human HCC tissues compared to the surrounding liver tissue with cirrhosis and chronic hepatitis, suggesting a role for NGF in the progression of HCC [108]. In fact, hepatic stellate cells express neurotrophins and their receptors are increased during hepatic regeneration [109, 110]. NGF and its related receptors play an important role in modulating the physiopathology of the intrahepatic biliary epithelium in the course of liver tissue remodeling processes and HCC progression. The mechanism of NGF involvement in liver tissue remodeling processes and HCC remains unclear. Rasi et al. [111], defining NGF distribution both inside the liver and in the intracellular compartments (in the cytoplasmic vesicle and in the endoplasmic reticulum), demonstrated that NGF can function in a paracrine and autocrine manner as a messenger molecule in the cross-talk between different cell types. An interesting perspective for the possible use of NGF is not only as a marker of progression and transformation but also as an attractive target for future therapeutic approaches [111].

Serum Proteomics

Serum proteomics, through the study of serum protein profiling, is useful in the detection of new biomarkers for early HCC diagnosis. Serum proteomics aims to identify the changes in protein expression, structure, and post-translational modifications. Some of these modifications are connected to HCC development. Recent studies have detected serum protein profile derived from patients with or without HCC. The serum of these patients is depleted of the most abundant protein as it has been shown using the proteomic analysis applying the method of surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDY-TOF MS) protein Chip system. Through this approach, 30 peaks have been detected and the levels of these were different according to the presence or absence of HCC. Particularly, a combination of six of these peaks distinguished HCC and non-HCC patients. The fragment C-terminal of vitronectin was identified as the highest discriminating peak (8,900 Da). Vitronectin is a

glycoprotein that is produced by hepatocytes and plays an important role in cell adhesion, migration, and matrix remodeling of cancer cells [112]. Since vitronectin gene expression is downregulated in HCC tissues, its increase is correlated to its own degradation. Additionally, in HCC there is an increase of metalloprotease-2 (MMP-2) gene expression and activity [113] that is involved in vitronectin catabolism. Therefore, in the serum protein profiling of HCC patients, the 8,900-Da biomarker may reflect tumor aggressiveness. A correlation was found between the 8,900-Da peak and tumor size. So, in the serum protein profiling of HCC patients, the 8,900-Da biomarker may reflect tumor aggressiveness.

Other Markers

β2-microglobulin (β2MG) serum concentration is increased in different chronic inflammatory and tumor diseases [114, 115]. The β2MG production by hepatocytes is associated with chronic inflammation correlated to viral hepatitis (hepatitis B and C virus) [116]. HCC cells show a higher expression of class I HLA antigens than normal hepatocytes, so tumor cells avoid immunological response [117, 118].

The β2MG serum concentration, which is increased in HCC, is correlated with class I HLA antigen expression levels. There is a positive correlation between β2MG serum concentration and interleukin-6 levels [119]. It seems that Il-6 is able to reduce immunological response and to induce the enhanced expression of β2MG in HCC cells. Moreover, β2MG serum levels are correlated with tumor size [120]. Therefore, it is considered a useful marker for indicating HCC progression. Glycylproline dipeptidyl aminopeptidase (GPDA) is consistently positive in patients with HCC [121]. This marker is particularly useful for HCC diagnosis in patients with non-AFP-producing HCC. Further studies are needed to establish the utility of these markers in clinical practice [122].

Summary and Perspective

Conclusions

The question of which molecular markers will prove to be the most useful for selecting treatment for individual patients with HCC and which will be validated remains unanswered. In this review, we have summarized the prognostic and predictive factors of these markers (Table 1).

Furthermore, we still do not know whether the molecular profile of a tumor changes at the time of disease recurrence after surgery, or even after therapy for more advanced disease. There is little information as to whether primary and metastatic tumors always share the same molecular profile, although there is some evidence for molecular discordance between early and metastatic disease. If this finding is shown to be a frequent occurrence, repeat biopsy with molecular profiling of fresh tissue might be required when treatments change, especially if the new treatments have a specific molecular target [123–126].

In summary, serological markers specific for HCC play important roles in this disease in the following aspects:

Table 1 Usefulness of principal hepatocellular carcinoma

HCC marker	Principal use
Alpha-fetoprotein	HCC early diagnosis, monitoring, and recurrence
Lens culinaris agglutinin reactive AFP (AFP-L3%)	HCC early diagnosis and prognosis (vascular invasion and intrahepatic metastasis)
Des-γ-carboxy prothrombin (DCP)	HCC early diagnosis and prognosis (early portal vein invasion and metastasis)
α-l-fucosidase	HCC early diagnosis
Glypican-3	HCC early diagnosis
P-aPKCι, E-chaderin, β-catenin	HCC prognosis
Human carbonyl reductase (HCR2)	HCC prognosis
Squamous cell carcinoma antigen (SCCA)	HCC early diagnosis
Serum proteomics	HCC early diagnosis
Golgi protein 73	HCC early diagnosis
Chromogranin A (CgA)	HCC prognosis and possible therapeutic treatment
Vascular endothelial growth factor (VEGF)	HCC prognosis (metastasis development)
Hepatocyte growth factor (HGF)	HCC prognosis and disease recurrence
Transforming growth factor-β (TGF-β)	HCC progression
Serum anti-p53	HCC prognosis (poor differentiation)
Nervous growth factor (NGF)	HCC prognosis and progression

Screening for early malignancy: *α-feto protein* is a unique marker that is used in clinical practice in combination with hepatic echography in the screening of cirrhotic patients to discover HCC, but other markers have been studied to reach an earlier diagnosis. Moreover, cirrhotic patients can show a transient AFP elevation that is associated with hepatocyte regeneration as a consequence of liver necroinflammation [127, 128]. Persistent AFP elevation is found in some of these patients. In this case *Lens culinaris agglutinin reactive AFP* (AFP-L3%), measurement may be of help in the HCC diagnosis. AFP-L3% is the product of α-1-6 fucosyltransferase; this enzyme is higher in HCC tissues than in peritumoral tissues [129]. Therefore, AFP-L3% is considered more specific than AFP in HCC diagnosis.

Des-gamma-carboxyprothrombin is a useful marker for detecting HCC in conjunction with AFP and ultrasonography liver.

Acting as a diagnostic aid for HCC: In the HCC diagnosis, other AFP and AFP-L3%, other markers can be used. *Des-gamma-carboxyprothrombin* is an abnormal prothrombin identified as a biomarker for HCC diagnosis. *Squamous cell carcinoma antigen* (SCCA) expression is more increased in premalignant dysplastic nodules than in HCC [130]. Smaller HCC show a higher SCCA expression than larger ones: decreased SCCA expression is correlated with progression of tumor size while increased SCCA expression in surrounding non-tumoral tissues of larger HCC is a marker for neoplastic transformation. *Serum proteomics* is used for the serologic recognition of protein profiles associated with cancer. Proteomic approach can accurately identify clinical HCC in cirrhotic patients. *Golgi Protein 73* is considered a possible marker for HCC; in fact, it shows a specificity of 75% and a sensitivity of 69%.

Determining prognosis in HCC: Des-gamma-carboxyprothrombin is increased in advanced HCC with portal vein invasion. It is considered a prognostic indicator able to predict rapid tumor progression and poorer prognosis. *Glypican-3* expression is less frequently observed in well-differentiated HCC than in moderately and poorly differentiated HCC.

GPC3-positive patients show a lower survival than GPC3-negative patients.

Vascular endothelial growth factor regulates positively tumor neovascularization. HCC patients with over-expression of VEGF have a lower survival rate.

The increase of *P-aPKC-ι* expression is correlated with more aggressive tumoral behavior: it is considered a prognostic factor for the survival of HCC patients. *Chromogranin A* is used to evaluate neuroendocrine differentiation of HCC and it may be of help in the therapeutic approach.

AFP-L3% expression is correlated with infiltrative growth type and poorly differentiated cancer while DCP expression is connected to intrahepatic metastasis and vascular invasion. The over-expression of hepatic *transforming growth factor β1* is found in HCC and is correlated with carcinogenesis, progression, and prognosis of HCC.

Maintaining surveillance following surgical removal of the primary tumor: Since HCC patients are prone to develop a second liver tumor, other markers other than AFP are proposed for the patients' follow-up. AFP-L3% measurement after treatment can be useful for understanding the prognosis and recurrence of HCC. VEGF is a possible tumor marker for metastasis in HCC.

Monitoring therapy in advanced HCC: *Hepatocyte growth factor* is considered a useful marker for evaluating the possible complications arising after curative hepatic resection.

Serum anti-p53 positivity is correlated with a poor prognosis and a shorter survival. It is used in the planning of HCC therapy [131]. *E-cadherin* and *β-catenin* are reduced in poorly differentiated cancer and their expression is correlated with metastasis development.

Technical issues are also important in this argument. At present, very few routine clinical laboratories have access to sophisticated molecular techniques, such as qRT-PCR, mutational analysis, FISH, and microarray, although most can do immunohistochemistry. However, standardized, optimized protocols and antibodies need to be applied in order to validate prospective validation; these technologies will also need optimization and standardization before being generally accepted as a valid decision-making tool.

Microarray is also an exciting technique, but probably it is not ready for entry into routine clinical practice until relevant validation studies have been done in many centers.

Ultimately, the most promising biomarkers of prediction and response require prospective validation in carefully designed randomized clinical trials using standardized protocols. This will require cooperation across borders and specialties.

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