



## REVIEW ARTICLE

## Virus induced hepatocellular carcinoma (HCC) and protein biomarkers

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## ABSTRACT

Hepatocellular carcinoma (HCC) is a standout amongst the most widely recognized cancers around the world, and just as the alcoholic liver disease it is also progressed by extreme viral hepatitis B or C. At the early stage of the disease, numerous patients are asymptomatic consequently late diagnosis of HCC occurs resulting in expensive surgical resection or transplantation. On the basis of the alpha fetoprotein (AFP) estimation, combined with the ultrasound and other sensitive imaging techniques used, the non-invasive detection systems are available. For early disease diagnosis and its use in the effective treatment of HCC patients, the identification of HCC biomarkers has provided a breakthrough utilizing the molecular genetics and proteomics. In the current article, most recent reports on the protein biomarkers of HBV or HCV-related HCC and their co-evolutionary association with liver cancer are reviewed.

## INTRODUCTION

The primary type of liver cancer which is common in adults is hepatocellular carcinoma which commonly results in death of people with cirrhosis. In various regions of Africa, China and Southeast Asia, the cancer which is rising at the fastest rate is the HCC [1]. In HCC, approximately 500,000 newly diagnosed patients arise every year [2]. To overcome the problems faced during the research of HCC clinically, a comprehensive knowledge of the disease characteristics and its epidemiology is very much required [3]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are majorly involved in causing HCC due to their persistent infections. HCC is a complex process having many multi-factors and steps. This explains its fifth position in the world of most common cancers and in terms of being the

deadliest it holds the third place [4]. The progression of HCC is rapid causing higher fatality rates. HCC is not always primary, it can also be due to cancer metastasis from other body parts and this is mostly in countries where hepatitis is not epidemic. Liver inflammation due to hepatitis virus causes hepatitis. Autoimmune diseases, toxic substances and other infections can also result in hepatitis. Out of 6 different hepatitis virus types which include A, B, C, D, E and G, the most common ones are A, B and C. Infected body fluids and blood transmit hepatitis B while hepatitis C is transmitted by being exposed to infected person's blood. For HCC patients, the only treatment available is hepatic resection or transplantation [5]. HCC at early stages has asymptomatic nature which proves to be a difficulty in the early stage detection and that is why when diagnosed, most people have the

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advanced stage and such patients recover very poorly. The potential protein biomarkers, their recent studies related to diagnosis of HCC are summarized in this review.

## 1. Hepatocellular Carcinoma Biomarkers

For various disease types, different molecular indicators can be used for disease identification and management. These molecular indicators can be easily detected in blood, tissue and urine samples and are referred to as biomarkers. Disease activation requires certain cofactors to be present which can be detected by different threshold concentrations of the biomarkers. Progression of a disease, its diagnosis and guide therapy are a result of the fluctuating threshold concentrations of the biomarkers. From disease detection to research, different biomarkers have been identified and employed. There are still studies being carried out to understand and evaluate the clinical purpose of biomarkers to their full potential and utilization. A great deal of money is used when broad treatment methods are used and comparing them with biomarkers, healthy amounts of money can be saved. Broad treatment can easily be shifted towards targeted therapy, simply by applying the biomarkers to measure and detect if the disease is present or not and its level of progression. Using these biomarkers, various types of cancer can be detected successfully as well as the hepatocellular carcinoma. Discovery of the molecular biomarkers involved different scientific platforms such as proteomic and genomics. Identification of these biomarkers uses some common assay techniques such as glycomics and metabolomics etc. During different levels of the disease progression, the screening of biomarkers is done by different techniques such as northern blot, microarray and gene expression etc. [6].

## 2. Protein Biomarkers

### 2.1 Alpha fetoprotein (AFP)

Alpha fetoproteins (AFP) for many years have been commonly screened in blood as a biochemical method to detect liver cancer. AFP is known to be a 70kDa glycoprotein, secreted by immature fetal liver cells and for its presence in cancer cells. For fatty acids, steroids, flavonoids, heavy metals, bilirubin and various other ligands, the transporter molecule involved is AFP [5]. AFP is an important component in cell division regulation and therefore, is considered to display immunosuppressive activity [7]. In the synthesis of AFP, liver and the yolk sac are involved. Adults have low levels of AFP while in the fetus it is considered as one of the chief proteins. When affected by cancer, a person exhibits high concentration of AFP in the blood thus making it a tumor marker. Mostly extreme levels of AFP are found in chronic hepatitis and cirrhosis patients [8]. In the case of hepatocellular carcinoma, early stages record increased alpha fetoprotein level which, during disease progression, returns to normal level. In benign hepatocellular carcinoma, lower AFP levels have been seen while generally, presence of HCC is indicates serum AFP level of 500ng/mL or more [5]. AFP-L1, AFP-L2 and AFP-L3 are three different glycoforms of AFP, out of which large cancer tissue mass, poor differentiation and malignant features link up to AFP-L3 [9]. Chronic liver disease and other benign pathologies can cause total serum AFP  $\leq 200$ ng/mL in individuals who can be differentially diagnosed using AFP-L3.

### 2.2 Des-gamma-carboxy prothrombin (DCP)

In 1984, DCP was discovered. Antagonist II (PIVKA-II) induced this abnormal prothrombin protein which in the case of liver cancer, increases in number [10]. For HCC cell lines, thrombogen presents itself as an autologous mitogen. During thrombogen formation, liver

carboxylation results in production of an abnormal product which is represented as DCP [11-12]. For HCC detection, studies showed that AFP and DCP combined gave more promising results than their individual usage [13].

### 2.3 Golgi protein 73 (GP73)

Normally residing within the golgi complex, a 73kDa transmembrane glycoprotein exists which is termed as GP73. GP73 is expressed in normal biliary epithelial cells but not in normal hepatocytes. In terms of HCC and other liver diseases, the expression rate of GP73 increases. HCC patients have a valuable biomarker in them which is the serum GP73 [14].

### 2.4 Neprilysin (CD10)

Present on the cell surface, consisting of 90-110 kDa, a metalloprotease enzyme exists, called as neprilysin (CD10) [15]. From their function in the nerve tissue of abnormal misfolding degradation of amyloid beta sheets to inactivating bioactive peptides, CD10 are employed. Due to their function in the nerve cells, they are also referred to as common acute lymphatic leukemia antigen (CALLA) [15]. For the differentiation of certain cells such as NK cells, B cells and T cells, CD10 is considered [16]. The presence of CD10 has been reported in immature B cells and on the surface of germinal center B cells inside the lymphoid tissue and the normal early lymphoid progenitor cells. In certain normal cells, neoplastic B cells and the precursor B cells, expression of surface proteins is due to a result of the abnormal methylation of CD10 [15]. The cells involved in the neoplastic development and progression experience an increase in their growth, migration and survival because of loss in CD10 [17]. In the case of hepatocellular and renal cell carcinoma, for the B cell expression, the CD10 is thought to be an important marker. Primary carcinoma has low expression of CD10 while the metastatic melanomas has more level of CD10 expression [18]. In HCC, the most common problem

encountered is the liver inflammation and its persistence. In immune response regulation, the immune cells are involved in the production of various cytokines, for which many genes are responsible. In activities regarding the body immunity, normal liver tissues play a vital role as they are responsible for increasing cytokines in the body which are required for the anti-inflammatory response and in the suppression of immune action [19]. Using fine needle aspiration biopsy of the liver, 55 HCC diagnosed cases in the United States were studied using the technique of immunostaining. Monoclonal antibodies were used to stain the sample against CD10 antigen. Results showed that 22 HCC diagnosed patients i.e. 86% gave positive canalicular staining results for CD10. Whereas for the cytoplasmic and membranous staining, 13% of the HCC diagnosed patients displayed positive results [9]. Liver tissues with mild fibrosis and inflammations displayed positive results for the canalicular staining of CD10 for 164 patients in the UK whose liver biopsies were done. However, as the fibrosis and inflammation increased the staining pattern of CD10 decreased [20]. 22 HCC diagnosed patients from India also showed 68% positive staining results for CD10 [21]. In Germany, immunostaining antibodies against CD10 were used for the differentiation of HCC and non-HCC patients by studying the CD10 expressions which revealed 63 HCC and 25 non-HCC cases [22]. CD10 thus, so far in differentiating HCC and non-HCC cases, has proved to be a very good biomarker [23].

### 2.5 CD36

CD36 is a protein of the membrane; it is present on the surface of the platelets as a fourth glycoprotein and is referred to as fatty acid translocase (FAT) [24-25]. From controlling inflammation, atherosclerosis, diabetes, to removing apoptotic cells, cell adhesion and in the transport of lipids, this multifunctional complex is involved in all of these processes [26]. Prostaglandin E2 production, activation of

phospholipase and calcium flux ion channels all are associated with CD36 as the recent studies reflect. Different cell phenotypes such as inducing production of foam cells, cytokines activation are differentiated by CD36 expression in tumor tissues [27]. Due to less CD36 expression, the immune system is attacked as the tumor antigens move into the cytotoxic T cells due to mediation of extracting apoptotic cells [28]. This proves that any change in the CD36 expression can induce carcinoma. Reaction of microvilli of hepatoma and melanoma cells with MO30, which was a novel antibody, helped to spot CD36 [29]. CD36 was seen to be associated with increase steatosis, hyper insulineamia and resistance of insulin by studying reports of CD36 expression in patients from Spain out of which 66 cases were HCV affected patients and 32 non diseases liver cases [30]. Deposition of liver fat in HCV affected patients was due to shifting CD36 fatty acid to the cell membrane [31]. Serum CD36 levels showed linkage with obesity and also showed the severity of CD36 expression on the Kupffer cells of the patients who have chronic liver disease related to HCV [32].

## 2.2 Glypican-3 (GPC3)

Glypican-3 is recognized as a GPC3 gene coded protein family member [33]. Several growth factors are regulated by GPC3 as it has the ability to stimulate or inhibit this action by signaling receptors. In the placenta and liver cells of the fetus GPC3 are normally expressed but adult liver tissue lacks this expression [34]. Serum of HCC patients showed higher levels of GPC3 but not in the case of benign liver disease and healthy donors which marked GPC3 as a relatively useful HCC tumor marker [35-36]. Tumor growth is stimulated by GPC3 by the activation of the Wnt signaling pathway [37]. Hepatitis patients have higher GPC3 positive percentage [38]. GPC3 was seen to be sensitive for HCC diagnosis using immunohistochemistry staining done for 80 cases from USA involving resection HCC, but the reports also showed that GPC3 is not effectively seen in

highly well differentiated hepatocytes and HCC variant fibro lamellar [34].

## CONCLUSION

The process of treating and managing hepatocellular carcinoma still proves to be a difficult task. HCC has a lot of molecular abnormalities as it develops and progresses. Recent studies show that different HCC patients show phenotypic differences between their tissues. Immunologically affected cells are replaced as a result of improved hepatocyte turnover, a feature which is common in HCC, HBV and HCV infections. Liver cancer chances are increased due to both HBV and HCV infections at such a rate that half liver cancer cases are due to HBV and one third liver cancer cases are due to HCV. It is difficult to differentiate HBV or HCV or both as the cause of HCC. Early diagnosis of HCC is therefore very important for correct and effective treatment. Several biomarkers for early diagnosis of HCC are present out of which the protein biomarkers were reviewed in this article. Finding the best biomarker is very difficult so a combination of 2-3 biomarkers is used for specific and sensitive results in the diagnosis of HCC.

## Conflicts of Interest

There is no conflict of interest among authors.

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