Chapter

Role of Biomarkers in Hepatocellular Carcinoma and Their Disease Progression

S.S. Haque, Ravi Bhushan Raman and Mehboobus Salam

Abstract

Hepatocellular carcinoma (HCC) is one of the third leading and common lethal cancers worldwide. Early detection of tumorigenesis of hepatocellular carcinoma is through ultrasonography, computerized tomography (CT) scans, and magnetic resonance imaging (MRI) scans; however, these methods are not up to the mark, so a search for an efficient biomarker for early diagnosis and treatment of hepatocarcinogenesis is important. Proteomic and genomic approaches aid to develop new promising biomarkers for the diagnosis of HCC at the early stages. These biomarkers not only help in prognosis but also provide better therapeutic intervention against HCC. Among the different biomarker candidates, liquid biopsy [including circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA)] has recently emerged as a noninvasive detection technique for the characterization of circulating cells, providing a strong basis and early diagnosis for the individualized treatment of patients. This review provides the current understanding of HCC biomarkers that predict the risk of HCC recurrence.

Keywords: hepatocellular carcinoma, biomarkers, diagnosis, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is the fourth most common type of primary liver malignancy and is prevalent in almost most parts of the world [1]. Hepatitis B virus or hepatitis C virus is the most important contributing factor [2]. Liver biopsy is invasive but still the gold standard for determining the presence of the tumor. However, in some cases, preoperative biopsy of the tumor samples does not accurately predict microvascular invasion when compared with the final specimen examination after liver resection [3, 4]. Due to the risk of needle tract tumor seeding and other limitations, preoperative biopsy is not currently recommended for routine HCC evaluation. Biomarkers are biological indicator molecules of physiological and disease states. The detection of HCC in the blood is simple, noninvasive, reliable, and the best choice for screening of HCC. In the coming year, newly identified biomarkers will be more thoroughly evaluated for the diagnosis of HCC (**Figure 1**). In this review, some promising biomarkers are discussed.

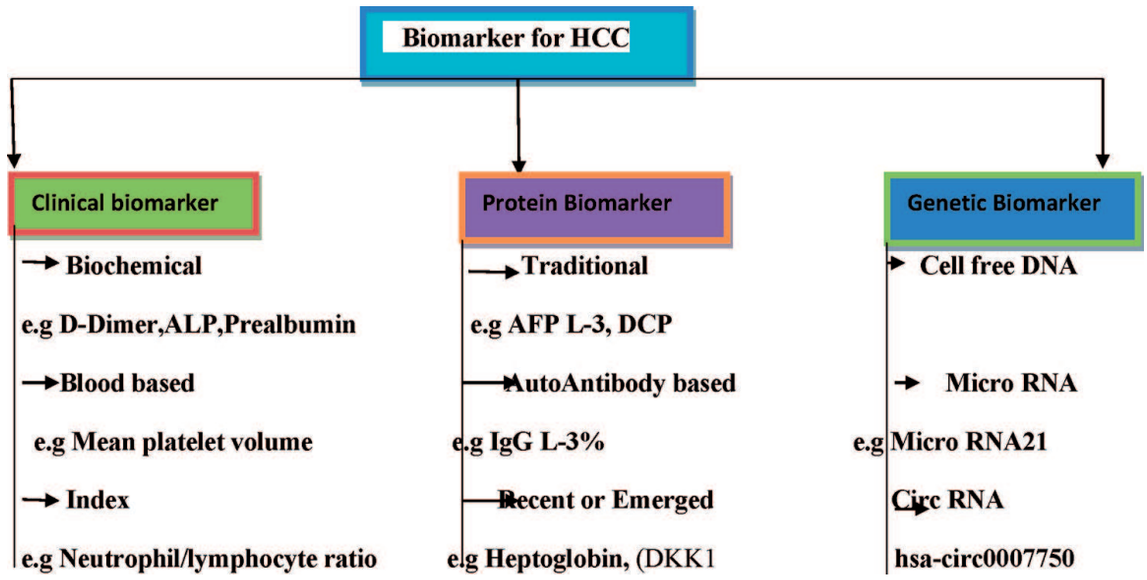

Figure 1.
Biomarker for HCC.

As a clinical biomarker, D-dimer is commonly used in HCC because portal vein thrombosis (PVT) is one of the important complications of HCC. D-dimer is the end product of fibrin degradation and is produced by fibrinogen, so D-dimer is a marker of endogenous fibrinolysis and its levels increase with fibrinolysis [5, 6]. Prealbumin (PA) or transthyretin is a homotetrameric protein that has been recently identified as a biomarker by the liver [7]. In contrast to albumin, prealbumin has a short biological half-life and reflects recent status [8]. Preoperative prealbumin level is useful for predicting long-term outcomes in patients undergoing liver resection for HCC.

The protein biomarker alpha-fetoprotein (AFP) is an important traditional diagnostic biomarker for hepatocellular carcinoma (HCC) for the past several decades. It is a 67-kDa glycoprotein that is produced by the liver in early fetal life when hepatocytes have not yet matured, and in HCC because of the dedifferentiation of hepatocytes to a more fetal pattern of gene expression. Serum AFP levels of more than 500 ng/ml are considered to have diagnostic significance; however, these values are reported only in a small percentage of patients with HCC. Many studies have reported that elevated serum AFP levels are correlated with increased risks of HCC in individuals infected with the hepatitis C virus (HCV) [9, 10]. In the practical aspects, the serum AFP concentrations do not correlate well with the prognostic values of HCC, such as the size of the tumor, stages, or disease progression, and ethnic variability may also be reported. Furthermore, in some cases of HCC, AFP elevations are not apparent at all and lack apparent discriminating power [11]. In HCC, the liver is damaged by one or more preexisting pathologic conditions, including cirrhosis and chronic hepatitis resulting from HBV or HCV infection [12], which also exhibited high serum AFP levels [13]. Three types of glycoforms of AFP have been identified so far, which are based on the difference in their binding affinities for Lens culinaris agglutinin (LCA). AFPL1 is a nonbinding fraction and AFP-L2 is a weakly binding fraction of total AFP. AFP-L3 is associated effectively with LCA [14], which is why it is a more specific biomarker for HCC. The AFP-L3 fraction has significantly increased the sensitivity for the early detection of HCC. However, AFP-based diagnostic approaches are still far from satisfactory results.

2. Serum autoantibodies

At an early stage of carcinogenesis, a small amount of tumor antigens can be produced by tumor cells that ultimately generate autoantibodies. These autoantibodies are stable in blood circulation and remain elevated for a long time [15]. IgG-L3% HCC-derived immunoglobulin G (IgG) and any type of unwanted glycosylations cause carcinogenesis. The fraction of Lens culinaris agglutinin-binding IgG (IgG-L3) among total serum IgG (IgG-L3%) increases with tumor load compared to healthy volunteers and asymptomatic HBV carriers.

3. Emerging serum protein biomarkers

Although traditional biomarkers have certain diagnostic values for HCC, none of them have been suitable in clinical practice, but there are emerging serum biomarkers. For example, aldo-keto reductase family 1 member B10 (AKR1B10) is a novel secretory protein that is associated with lung, breast, and colorectal cancers but induced in hepatocellular carcinoma (HCC), and this protein is located on chromosome 7q33 [16, 17] and is one of the important diagnostic and prognostic biomarkers for HCC [18–20]. AKR1B10 protein is an oncogenic protein that induces tumor development and progression by the removal of cytotoxic carbonyl compounds [21–23]. Dickkopf-1 (DKK1) is a 266-amino acid (35-kDa) secreted glycoprotein and antagonist of the Wnt/beta-catenin pathway signaling pathway discovered in 1998, which is expressed in a variety of human tumors. It is one of the impaired signaling pathways in hepatocellular carcinoma (HCC). Its function seems to be contradictory in the process of tumorigenesis, acting either as an oncogenic promoter of metastasis or as a tumor suppressor. In many tumors, high expression of *Dkk1* may promote tumor metastasis. However, *Dkk1* can inhibit tumor invasion and metastasis [24–26] and plays an important role in regulating human HCC cell migration, invasion, and tumor growth [27]. Therefore, novel genetic biomarkers for early diagnosis and detection are needed.

With the advent of cellular and molecular techniques for a better understanding of tumor biology, the role of biomarkers related to early detection has attracted a great deal of research interest resulting in the discovery and utilization of several novel markers in this disease. Liquid biopsy is one of the potential and noninvasive procedures that have attracted much attention to identify tumor markers in peripheral blood for diagnosis, monitoring, and prognosis of cancer, and overcoming tissue biopsy limitations. In this procedure, the sampling and analysis of biological samples, such as blood, urine, saliva, or stool, are done where nucleic acids originating from all or part of the body can be found including circulating tumor DNA (ctDNA) or RNA, exosomes, and circulating tumor cells (CTC). Most of the studies are carried out for mutations of ctDNA to detect minimal residual disease, as diagnostic markers or response to therapy. For the mapping of genetic alterations in ctDNA either next-generation sequencing (NGS) or digital PCR are used. Circulating tumoral cells and tumoral cell-free nucleic acids in peripheral blood could signal the presence of micrometastasis, and their utility has been explored in HCC diagnosis and prognosis.

Some other circulating RNAs have been explored, but none of them have been widely recognized as valuable markers of HCC recurrence, probably because none of them are specific for HCC [28]. In cancer biology, different types of circulating cellular elements have been identified as tumor markers [29, 30]. One of them is

circulating tumor cells (CTC), which consist of obtaining a sample in a convenient and minimally invasive manner at multiple time points over the course of the disease.

4. Circulating tumor cells (CTCs)

Circulating tumor cells (CTCs) are defined as the primary solid tumor cells shed into blood, bone marrow or lymphatic vessels, or other healthy organs. These cells have a strong potential for distant metastasis, circulating through the bloodstream, and traveling to different tissues or organs of the body [31]. This type of tumor development process occurs at every stage. Australian doctor Thomas R. Ashworth was the first to discover CTCs in 1869 in the blood of a breast cancer patient [32]. CTCs are one of the useful markers for early diagnosis and monitoring of disease relapse. Their concentration is very low in blood, which limited the studies on CTC. Metastatic cells struggle to survive in the bloodstream and less than 0.01% of CTCs introduced into the circulation survive to produce metastases [33]. In recent years, with the advent of new technology, the separation and enrichment of CTCs have been greatly improved. In cancer, CTCs have received great attention in trying to estimate the future course in patients with breast cancer, colon cancer, and prostate cancer.

CTC analysis might provide personalized and effective strategies for clinicians and researchers because CTCs are sensitive biomarkers that enable early diagnosis, real-time monitoring, and molecular characterization to facilitate the implementation of precision medicine. In addition, the different study shows powerful evidence for the potential clinical value of the CTC assay [34]. However, numerous obstructions must be overcome before CTC analysis can be applied in the clinic. The CTC detection methods mentioned above have their own advantages and drawbacks. It is one of the important tasks to establish a highly sensitive and specific method that provides the full spectrum of CTCs. Therefore, the standardized method for CTC evaluation includes sample preparation, enrichment, and detection. In addition, lots of studies are single-center case-control research, with limited sample sizes. Validation is sometimes difficult or impossible to achieve. A multicenter prospective study with sufficient sample size and long follow-up is needed for CTC detection methodologies, the detection method must be uniform, and large samples can provide powerful validation for accurate analysis and standard evaluation of the final data. Although CTC detection is currently used in research, technological advancement will make it feasible in clinical practice in the near future.

5. Circulating tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) has attracted extensive attention for its wide application in cancer research, and it consists of mutant DNAs or tumor-derived fragmented DNA released into the circulation by tumor cells and constitute part of circulating cell-free DNA (cfDNA) in different types of cancer [35], cfDNA levels vary substantially from <0.01% to >60% of alleles in circulation [36, 37]. ctDNA carries the genetic information of the tumor, and it is highly specific and can detect at very low concentrations, making it suitable for early diagnosis, treatment, and monitoring of tumor progression. ctDNA is a liquid biopsy to profile the genome of a tumor more comprehensively than conventional sampling methods. Thus, it is an important tool to provide information for guiding targeted therapy [38], unveiling drug resistance [39], and monitoring treatment

response [40]. Analysis of ctDNA helps in the efficient evaluation of disease status and early detection of recurrence, providing an average of 10–12 months' lead time for detection of metastatic recurrence compared to traditional modalities [41]. After resection, detectable ctDNA could identify cancer patients at high risk of recurrence, [42] while dynamic ctDNA changes can predict clinical relapse [43]. ctDNA provides information about specific tumor genes, such as DNA methylation, point mutations, copy number variations (CNVs), and chromosomal rearrangements. It also provides a unique opportunity for serially monitoring tumor genomes in a noninvasive, convenient, and accurate manner. Two different changes are monitored during the detection of ctDNA—quantitative changes and qualitative changes. The first detection method measures the quantity of ctDNA in circulation, and the second detects tumor-specific genetic aberrations. Many studies have carried out quantitative changes in cfDNA in the blood of HCC patients and show that elevated levels of cfDNA may represent an important complementary tool with potential clinical applications for detection, screening, treatment monitoring, and predicting metastatic potential [44–51].

Cell-free nucleic acids (cfNA) were first reported in human peripheral blood by Mandel and Metais in 1948 [52]. However, their studies did not recognize until 30 years later with the discovery of higher concentrations of cell-free DNA (cfDNA) in serum and plasma from cancer patients compared to healthy individuals. Currently, cfDNA is supposed to be released by normal cells at an average concentration of 30 ng/ml (0–100 ng/ml) into peripheral blood at the physiological level [53]. The concentration of cfDNA was accompanied by a decrease in DNase activity because cfDNA is degraded by peripheral blood deoxyribonuclease activity. Normal cells in peripheral circulation can also release cfDNA, and this reduces ctDNA concentrations [54]. cfDNA lysis occurs secondary to the clotting process of blood cells in collection tubes; thus, several studies have found significantly high cfDNA concentrations in serum than in plasma [55, 56]. Similarly, the blood specimen collected improperly or mechanical shearing leads to the destruction of the blood cells, causing the release of cfDNA into plasma [57]. Until recently, many researchers preferred plasma fraction over that in serum for cfDNA analysis [58]. Although DNA in the plasma is least contaminated with blood cells, the amount of DNA in plasma is more or less affected due to the time interval between analysis and blood collection [59].

A large number of hypermethylated genes, such as developing brain homeobox protein 2 (DBX2) [60], G-protein-coupled bile acid receptor (TGR5) [61], metallothionein 1M (MT1M), metallothionein 1G (MT1G) [62] and I the cyclin kinase inhibitor (NK4A) [63], were detected as cfDNA from HCC patients and were identified as biomarkers of vascular invasion. In the process of HCC diagnosis, high degree of methylation at multiple genes has been shown to play an important role. In addition, to improve the diagnostic efficiency, the combined detection of the methylation status of multiple genes may be effective [64].

The presence of cell-free DNA in plasma/serum has been used to reveal tumor-associated biomarkers, such as the increased plentiful of cell-free DNA (cfDNA) in cancer patients or the presence of epigenetic or specific genetic alterations, which have been discovered in numerous types of cancers, including HCC. Many studies have reported that the cfDNA is a source of HCC biomarkers in the diagnostic and prognosis of HCC in clinical settings also; HCC-specific biomarkers should be validated to determine their association with HCC recurrence. Finally, different micro (mi) RNA signatures in liver tissue have been associated with HCC [65, 66]. However, the necessity of obtaining liver tissue samples limits their application preoperatively, and circulating miRNAs are at present being explored. Several circulating miRNAs

have been reported as important biomarkers for HCC diagnosis [67], prognosis, and vascular invasion [68, 69]. To date, there are no data about the association of miRNAs with HCC recurrence, and future studies are needed to explore the utility of these promising biomarkers.

6. Circulating microRNAs

Circulating microRNAs (miRNAs) were first proposed as potential cancer biomarkers in 2008 [70]. Blood miRNAs, which circulate in a highly stable, cell-free form, show promise as novel potential biomarkers for early detection of HCC. A number of evidence indicate that these noncoding nucleotide sequences are resistant to RNase degradation, repeated freeze-thaw cycles, boiling as well as acid/base treatment [71, 72]. The stability of circulating miRNAs has attracted attention from clinical researchers, who want to investigate their diagnostic utility for a wide range of diseases including HCC.

Under extreme conditions, their stability and their abundance in sera made circulating miRNAs as promising biomarkers for cancer pathologies [73, 74]. Serum miRNAs are not digested by ribonuclease because they are encapsulated in protein complexes or in membranous microvesicles that transport them in the circulatory system [75, 76]. The circulating miRNAs are stable when the samples are stored at −80°C [77]. Despite the valuable investigation of extracellular miRNAs, the use of miRNAs as biomarkers of cancer is still regarded as a "work in progress" and mostly confined to research programs [78]. Continuing technological advancements, however, like second-generation sequencing, as well as a perceptive of the pathobiological role of miRNAs, emphasize their future promise as clinical biomarkers [79].

7. Circular RNAs

Circular RNAs (circRNAs) are covalently closed, single-stranded, and stable RNA molecules [80] that have been evaluated for the diagnosis of various cancers. They contribute importantly to gastric cancer [81, 82], breast cancer [83], lung cancer [84, 85], pancreatic cancer [86], and HCC [87]. In a multicenter study, three circRNAs (hsa_circ_0000976, hsa_circ_0007750, and hsa_circ_0139897) were successfully validated in the plasma of the hepatitis B virus-related HCC patients, and their plasma levels positively correlated with HCC relapse, while after hepatectomy their levels decreased [87].

8. Conclusion

In the present scenario, no single or biomarker combination is predictable enough to diagnose a HCC lesion without confirmatory histological or radiological data. None of the new tumor markers excel the conventional ones in such a way that it has been widely endorsed in clinical practice. The liquid biopsy is one of the critical parts of precision medicine, and it is likely to be useful in the near future. However, future research should develop useful HCC biomarkers for monitoring treatment activity, detecting early resistance to treatment, and identifying patients who would more likely benefit from treatment.

We expect that identifying novel cost-effective and high-efficient biomarkers for the early diagnosis of HCC will be promising.

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