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The search for biomarkers of hepatocellular carcinoma and the impact on patient outcome

Alyson P Black and Anand S Mehta

Hepatocellular carcinoma (HCC) is the 5th most common cancer, but the 3rd leading cause of cancer death globally with approximately 700,000 fatalities annually. The severity of this cancer arises from its difficulty to detect and treat. The major etiologies of HCC are liver fibrosis or cirrhosis from chronic viral infections, as well as metabolic conditions. Since most cases arise from prior pathologies, biomarker surveillance in high-risk individuals is an essential approach for early detection and improved patient outcome. While many molecular biomarkers have been associated with HCC, there are few that have made clinical impact for this disease. Here we review some major approaches used for HCC biomarker discovery — proteomics and glycomics — and describe new methodologies being tested for biomarker development.

Address
Medical University of South Carolina, Charleston, SC 29425, United States

Corresponding author: Mehta, Anand S (mehtaa@musc.edu)

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Introduction

Hepatocellular carcinoma (HCC) is a malignancy of hepatocytes that arises within the liver. This cancer occurs in the background of patients with underlying liver disease such as liver fibrosis and cirrhosis often associated with chronic viral infections. Additionally, obesity-associated nonalcoholic fatty liver disease/nonalcoholic steatohepatitis has been recently considered a major etiology of HCC [1•]. The survival rate of people with primary liver cancers is very low, with a 0.95 ratio of mortality to incidence [1•]. The low survival rates have been attributed to late diagnosis and limited treatment options [2]. Although liver transplantation is the preferred option for surgical treatment of HCC, the paucity of organ donors means that partial hepatic resection is a common treatment [3]. Unfortunately, even with advances in surgery and patient care, reported 5-year survival rates are around 50% [4]. HCC is consequently responsible for approximately 700 000 deaths annually and ranks as the 3rd leading cause of cancer death worldwide [3,5]. The incidence of HCC has shown a drastic increase in the United States over the last 35 years [6], mainly attributed to hepatitis C virus infection and rising obesity/metabolic challenges [1•].

Treatment of HCC

As a highly lethal cancer, successful treatment options for HCC are few. According to the American Association for the Study of Liver Diseases treatment guidelines for HCC, surgical resection or ablative strategies can be therapeutically valuable options for patients with small lesions and well-managed liver disease [5]. Candidates for resection are those without severe cirrhosis and who have 1-3 unilobar lesions (<3 cm for multiple lesions or <5 cm for one lesion), and this therapy is recommended over radiofrequency ablation [5]. Unfortunately, only about 10% of HCC patients are acceptable for resection [3], and there is significant risk of recurrence or *de novo* tumor development following the resection or ablation of HCC lesions [4]. The most effective treatment option for HCC patients is liver transplantation, as it rids the patient of both the cancer and the underlying liver disease. Transplantation thus provides the best outcomes for patients, with 5-year survival rates of 70% and low risk of recurrence [4]. However, the major limits to liver transplantation are the shortage of organ donors as well as the stringent criteria for transplantation [3]. Even though liver transplantation is often viewed as a cure for HCC, intra-hepatic tumor recurrence can occur and is especially a risk for those patients with large initial tumors [7]. Chemotherapeutic options for HCC are limited and the frontline agent for those with non-ablatable tumors is the multi-kinase inhibitor sorafenib, sold under the brand name Nexavar. Sorafenib is a general tyrosine and serine/threonine protein kinase inhibitor with activity against vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors as well as intracellular kinases B-Raf and Raf-1 [8]. Agents that specifically target one growth receptor, such as enhanced VEGFR inhibitors have failed to show activity against HCC [8]. It is noted, however, that sorafenib's activity against HCC is limited, with improved survival times of only a few months [9]. These bleak treatment options — both in their availability and efficacy — highlight the necessity for early detection of HCC.

Clinical detection of HCC

The current clinical gold standards for detection of HCC are magnetic resonance imaging (MRI), ultrasound (US), and computed tomography (CT) scans to detect lesions. However, a retrospective analysis performed in 2011 indicates shockingly low sensitivity of US to detect small lesions of HCC, with sensitivity being improved upon the addition of MRI and or CT scans [10]. The proposed sensitivity levels of US, CT, and MRI were 46%, 65%, and 72% [10], which are far below commonly desired values for a clinical biomarker. This highlights a disconnect between current clinical practice and the expectations for biomarker performance in clinical trials. A prognostic biomarker is a biological molecule that can predict the occurrence of a disease state — often before any noticeable lesion or physical abnormality may arise, creating significant pressure on biomarkers to indicate what is to come. Thus, the commonly held view of biomarkers as stand-alone clinical tests for early detection may be unrealistic. However, combining current clinical modalities with prognostic biomarkers could have significant benefit for detection, and a surveillance program study found that US screening combined with the glycoprotein biomarker alpha-fetoprotein (AFP) significantly increased the sensitivity of US screens from 43.9% to 90.2% [11]. The combination of US and AFP is now one of the most widely-used screening methods for HCC [10,11]. Along with prognostic biomarkers for detection, predictive biomarkers for HCC are also needed to suggest an individual's response to treatment. These predictive biomarkers could serve to assist clinicians in selecting appropriate candidates for liver resection/transplantation as well as predicting disease recurrence [4]. Described below are two current techniques for identifying biomarkers of HCC: proteomics and glycomics. Multiple markers have been observed via each method, yet their clinical impact is little to none at present.

Proteomic identification of biomarkers of HCC

The liver secretes many proteins into the blood, allowing for non-invasive collection of proteins for analysis. Various proteomic methodologies have been proposed to identify proteins that are altered in the serum of those with HCC, and most have involved the comparative analysis of several patient groups: healthy subjects, those infected with hepatitis B or C, those with liver cirrhosis, and those with both liver cirrhosis and HCC. By utilizing sensitive machines and methods, often some form of mass spectrometry or liquid chromatography, low abundance proteins that change with cancer development can be found and related to the cancer. Using such methods, proteins such as peroxiredoxin 3, osteopontin, and AFP have been identified as potential markers of HCC, with upregulation of these proteins observed in HCC patient samples compared to healthy individuals or those with liver disease [12–15]. As mentioned previously, AFP is currently used as a biomarker in the clinic alongside ultrasound, yet it lacks the specificity and sensitivity to stand alone as a powerful biomarker. Another serum protein that has shown potential as a biomarker is des-gamma carboxyprothrombin (DCP), and studies suggest it to be a more powerful biomarker than AFP for larger tumors as well as those arising from viral etiology [16,17]. Recent experiments have begun to utilize combinations of protein markers to create more sensitive biomarker panels, for example combining AFP with another serum protein, fibronectin 1 [18]. This multi-marker panel approach illustrates that detection performance can be improved by integrating separately characterized protein biomarkers.

Glycomic identification of biomarkers of HCC

Glycomics is the profiling of glycans (sugar structures) attached to larger molecules such as proteins. The variety of glycan structures that may be attached to a protein creates a post-translational diversity often ignored. There is significant evidence illustrating that glycan structures are altered in the presence of cancer [19•,20], and thus there is great potential for glycomic biomarkers as cancer-specific alterations attached to normal serum proteins. In regard to liver cancer, glycomic methodologies have long been used to either improve or discover biomarkers of liver cancer. Initial work showed that AFP with an attached α1,6 core fucosylated glycan was a better marker of HCC than AFP alone, and it became a USFDA approved biomarker known as AFP-L3 [21,22]. There is now substantial evidence to suggest that increased fucosylation occurs directly in the tumor and also that it plays a role in cancer development [23], Figure 1. One major issue with AFP-L3 is the protein to which the glycans are attached. That is, total AFP has a sensitivity of ~40–60%, a value which is not improved by the examination of the fucosylated glycoform [16]. Glycoforms are just a subset of the total AFP protein level, thus the sensitivity is not necessarily improved. However, as the results with AFP-L3 indicate that fucosylation is a highly specific HCC modification, groups have combined this glycomic information with proteomics to identify other proteins with glycan changes that could be used as biomarkers of liver cancer [24–29,30••].

The importance of glycosylation in HCC progression has been observed with α1,6-fucosyltransferase (FUT8), the enzyme responsible for catalyzing core fucosylation of N-glycans. Experiments with FUT8 knockouts showed significant reduction in growth factor signaling via the epidermal growth factor (EGF) and hepatocyte growth factor (HGF) receptors, as well as inhibited tumor formation in mice [23]. Additionally, a recent genomic analysis of HCC showed overexpression of the *FUT8* gene, highlighting the likelihood for increased core fucosylation to be found on glycoproteins of HCC patients [31••]. These data suggest that specific glycan alterations are key