Chapter

Translational Perspective in Hepatocellular Carcinoma

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Abstract

The burden of liver cancer is higher in Hispanics, African Americans, and Asians. Viral hepatitis (Hepatitis B and Hepatitis C viruses), non-alcoholic steatohepatitis (NASH), and alcoholic liver disease (ALD) are the most common etiological/risk factors for liver cancer. Approximately 80–90% of hepatocellular carcinoma (HCC) occurs in patients with underlying liver cirrhosis. Individuals with advanced cirrhosis represent a high-risk group for liver cancer. To fill the increasing gap between basic science and clinical research, translational research has been developed as an emerging technology. Basic science attempts to unravel the mechanisms of disease using tools (e.g., culture systems and animal models) that allow for easy manipulation of biological processes. Further, culture systems and animal models are useful to derive causal associations, but they generally do not include an endpoint directly applicable to clinical practice. Hence, development of new tools for early detection, including the evaluation of liquid biopsy, identification of tissue biomarkers of treatment response, execution of precision and enhancement of patient stratification in patients at risk for HCC development to enable chemoprevention clinical trials becomes important. It was identified as translational research has begun as an effective approach to facilitate the development of novel molecular-based biomarkers and to accelerate the implementation of laboratory discoveries into clinically applicable tools. Despite great advancement in diagnosis and management of HCC, the exact biology of the tumor remains poorly understood generally limiting the clinical outcome. Comprehensive analysis and characterization of the molecular mechanisms and subsequently individual prediction of corresponding prognostic traits would transform both diagnosis and treatment of HCC and is the key goal of modern medicine. To overcome the challenge and to accelerate the progress, a collaborative effort from various clinical research groups and translational approach is needed.

Keywords: biomarkers, clinical research, cancer, hepatocellular carcinoma and translational research

1. Introduction

Hepatocellular carcinoma (HCC) is a common form of liver cancer associated with high mortality rate [1]. It is estimated that approximately 750,000 new cases of HCC diagnosed per year worldwide which makes HCC as the fifth common cause of cancers affecting human [2]. Mortality rate of approximately 700,000 has been estimated annually due to HCC and it has been considered as the third common cause of death [3].

World Health Organization (WHO) reported that about one million people annually was diagnosed with HCC [4]. The major risk factors for developing HCC are viral infections, alcoholic liver diseases and nonalcoholic steatohepatitis (NASH) [5]. In most of the cases, HCC was diagnosed after the disease progresses, when survival rates are low [6]. Development of HCC is asymptomatic at early stages of the disease when current curative therapies are available [7]. Diagnosis of HCC is based on the combination of radiological, serological and histopathological criteria [8]. Almost 90% of the cases are diagnosed without the help of liver biopsy, as many non-invasive techniques such as serological examination and imaging techniques are used as standard diagnostic test for HCC [9]. Ultrasonography is the most widely used imaging test for screening because of its diagnostic accuracy, non-invasiveness, good acceptance by patients and moderate cost [10].

Because of a large variability in etiological and genetic backgrounds and the long-time development of the disease, HCC lesions are known to exhibit substantial intra-tumor and inter-tumor heterogeneity [11]. For the treatment stratification in HCC, tumor heterogeneity poses a significant challenge [12]. Non-invasive assessment of several tumor characteristics, such as cellularity, perfusion and oxygenation, can be performed using quantitative functional multiparametric magnetic resonance imaging (mpMRI), which can be also used for tumor characterization and for assessing the treatment response [13]. Although pathological and genetic heterogeneity in HCC lesions have been defined, imaging reports on HCC heterogeneity are extremely inadequate, with only one study reporting visual assessment of HCC heterogeneity on contrast-enhanced MRI, with no such study describing quantitative imaging measurements of HCC heterogeneity [14].

Nowadays, magnetic resonance imaging (MRI) analysis has been used to study the characterization of HCC lesions in biopsied or resected samples, and also it provides further information on tumor properties [15]. Without histological confirmation, HCC can be detected using imaging alone in most of the patients, [16], but histopathologic assessment has its own advantage over imaging analysis. Results of the study by Hectors et al. indicated that HCC patients could ultimately benefit from knowledge of about the correlation of imaging parameters with histopathological and genomics properties of HCC lesions [17].

Based on tumor burden, hepatic function and performance status, patient prognosis and treatment decisions are made [18]. Surgical resection and liver transplantation are generally recommended for HCC, but is indicated specifically for patients with early stage and well-preserved liver function [19]. For patients not suitable for curative treatment, transarterial chemoembolization (TACE) and transarterial radioembolization (TARE) may provide better loco-regional tumor control and increase patient survival [20]. It is commonly acknowledged as a palliative treatment option and improves survival in unresectable HCC.

Primary liver cancer or HCC imposes significant challenges to healthcare with huge unmet clinical needs. In males, it is the second leading cause of cancer-related mortality worldwide and 80% of HCC cases are found in the Asia-Pacific regions [21]. Although treatments such as surgical resection, liver transplantation or radiofrequency ablation are potentially curable options for early-stage HCC, recurrences remain the most common issue and limit the overall survival [22]. Survival may be prolonged by loco-regional therapy in intermediate stage HCC and by systemic therapies in advanced HCC [23]. Overall, when compared to other common cancers, clinical outcomes in HCC remain poor due to the lack of effective therapies [24]. It's intrinsically quite challenging to understand the biology of HCC as it is the common end-point of a number of etiologies with different molecular pathways. In addition to increasing the complexity, significant heterogeneity is also existent within the same tumor [25].

Treatment outcomes for the majority of patients with HCC have remained poor through the years. The overall 5-year survival rate for all patients with HCC has remained steady at 3–5% [26]. This results from two facts. The first major reason is that for most patients, diagnosis is made only when the disease is in advanced stage and inoperable, and the second reason is when the disease has very poor prognosis. Furthermore, cirrhosis of the liver is a major risk factor for HCC development [27]. In most of the cases, the cause of cirrhosis is mainly due to chronic hepatitis B or C virus infection, or heavy alcohol usage [28]. Other known risk factors may include hereditary hemochromatosis, α-1 antitrypsin deficiency, primary biliary cirrhosis, autoimmune hepatitis, smoking and aflatoxin exposure [29]. Regardless of the cause, the accompanying cirrhosis can independently cause death as well as complicate other treatments [30].

Because of the challenges faced by current and future populations due to HCC, treatment has been an ever-rising area of interest for research. So far there was no such improved results from cytotoxic therapies have been reported. Researchers' recent efforts have been mainly focused on a variety of proven tactics and techniques [30]. Reducing morbidity and mortality are the major concerns in the modern surgical era, as various studies have recommended that precise evaluation of liver function reserve is indispensable for prognosticating the occurrence of morbidities and mortalities [31]. A number of confounding variables and background liver changes pose a major challenge in clinical proteomics studies that target liver diseases and biomarker discovery. Fat accumulation, inflammation, necrosis, apoptosis, proliferation, fibrosis and viral replication can all occur simultaneously in liver injury [32]. In general, insulin resistance is associated with the pathogenesis of nonalcoholic fatty liver disease (NAFLD). A role of hepatic steatosis in the pathogenesis of chronic hepatitis C has also been studied, implying hepatitis C as a metabolic disease. As a result, there is a need for novel strategies and careful experimental design [32].

To improve our better understanding of the liver biology, integrative studies such as proteomics and basic cellular biology or other developing fields such as imaging studies and mouse models will play the most prominent role. The translation of basic discoveries into daily clinical practice will accelerate the ability to understand the underlying molecular dysfunction in human disease (such as signaling pathways, protein–protein interaction networks) [32]. A study by Li et al. showed how mouse models of liver disease can be used to provide valuable functional information. Therefore, it helps to improve the current concepts for better screening and prevention [33]. Conde-Vandellis et al., described that biomarker discovery revealed a powerful new path to study proteomic analysis of extracellular, circulating or urinary vesicles [34].

2. Biomarkers in HCC

Various circulating markers and tissue markers have been identified. Because of their low predictive accuracy and/or high cost, few biomarkers are acceptable for clinical utility [35]. Alpha-fetoprotein (AFP) was the first serologic assay for the detection and clinical follow-up of patients with HCC, which has been the standard tumor biomarker for HCC for many years [36]. Analysis of recent studies has indicated that AFP testing lacks adequate sensitivity and specificity for effective surveillance [37]. AFP levels were reported to be normal in up to 40% of patients with HCC, particularly during the early stage of the disease [38]. The combinational use of different biomarkers may enhance the detection sensitivity for the early detection of HCC. The tumor markers are most useful if utilized not only as confirmatory tests, but also as a part of routine follow-up [39].

Instead of simply utilizing a tumor marker test during the initial cancer evaluation, following the tumor marker levels serially against the background of clinical and other diagnostic findings will enhance the value of the tumor marker in providing information that could be used in therapeutic decisions and evaluation [39]. In recent years, many promising candidate biomarkers for HCC have been identified, but most of them have not been applied in the clinical diagnosis due to their limited practicability and high cost.

Discovering novel biomarkers that provide sensitive and specific detection of early stage disease when it is highly treatable is crucial [40]. In blood, the presence of low abundance and low molecular weight proteins and metabolites provide a potential and beneficial information, which also have great promise as a source of new biomarkers [41]. Unfortunately, they comprise less than 1 percent of the blood molecules, and in many cases exist in at two molar concentrations. The remainder of the proteins and peptides comprising the complex circulatory proteome range from 10 to 12 mg/mL to 10–3 mg/mL, spanning ten orders of magnitude, with a few high molecular weight proteins such as albumin and immunoglobulins accounting for 90% of total protein content [42].

It is quite difficult to detect and quantify low abundance, low molecular weight proteins and metabolites using conventional protein detection methods such as two dimensional gel electrophoresis (2-DGE), as they do not have the sensitivity and high resolution [43]. As the input volume is only a few microliters, it's also quite challenging for the moderately high-detection and sensitive modern mass spectrometers (at to molar concentration). And the complexity of protein mixture will influence the sensitivity and resolution [43]. The usual sample preparation steps for mass spectrometry (MS) experiments have several steps. MS experiments start with the depletion of high abundant proteins using commercially available immunoaffinity depletion columns. After depletion process, using size exclusion chromatography, ion exchange chromatography and/or isoelectric focusing it has been fractionized. However, as it has been recently shown that the vast majority of low abundance biomarkers are non-covalently and endogenously associated with the carrier proteins that are being removed, removal of abundant native high molecular weight proteins can significantly reduce the yield of candidate biomarkers [44]. Methods, such as size exclusion ultrafiltration under denaturing conditions, continuous elution denaturing electrophoresis or fractionation of serum by means of nano-porous substrates, have been proposed to solve this problem, but these methods are very time consuming, [45]. In the past, there has been no routine method for purifying and enriching low molecular mass peptides and metabolites from complex protein mixtures and biologic fluids in solution [46].

3. Translational research

In hepatology, HCC is an area that could benefit from translational research. In advanced-stage HCC, until 2016, sorafenib was the only systemic agent that can increase survival in patients [47]. In the meantime, four drugs, which include lenvatinib, regorafenib, cabozantinib and ramucirumab, have shown clinical efficacy either in first- or second-line therapy after phase 3 clinical trials [48]. Response rate of 14% for nivolumab and 17% for pembrolizumab was observed and they also increased the duration of response more than 1 year in half of the responders prompted the US Food and Drug Administration (FDA) approval of these two drugs under the accelerated program after single-arm phase 1/2 trials [49].

There are no other biomarkers except AFP that can identify the best responders to any other therapies, while the patients receiving ramucirumab in second-line

therapy [48]. To identify an immune subclass in HCC resection specimens, translational research efforts played a prominent role. Its ability to predict response to immune-based therapies is still under investigation [50]. This is quite different with other tumor types where comprehensive molecular profiling of large sets of samples enabled the identification of robust predictive biomarkers of treatment response (e.g., BRAF mutations and response to vemurafenib in melanoma, and ALK rearrangements and response to crizotinib in lung cancer) [51]. These translational research initiatives helped coin the concept of "oncogene addiction," a term that describes a selective dependence of cancer cell growth for a certain genetic alteration. Some of these biomarkers are included in clinical practice guide-lines and FDA label [52].

As translational research established itself as a bridge between basic research and clinical practice, its application spread beyond cancer to disease in general and then to non-biomedical fields such as engineering [52]. Genome-wide association studies have shown that only a small fraction of an individual's risk for cancer can be predicted by their genetic constitution and that hundreds of genetic variants conspire to determine that risk [53]. Often, disease-related genetic variants do not alter protein-coding regions of the genome, and evidence is emerging to show that they influence cell physiology by altering non-coding RNAs with gene regulatory roles [54]. Additional layers of complexity have emerged from the sequencing of cancer genomes. These efforts have revealed large intra-individual heterogeneity in neoplasms of the same organ and histotype, i.e., each tumor has its own mutational profile [54]. Additionally, they have uncovered substantial intra-tumoral heterogeneity that complicates treatment decisions and calls into question the strategy of genotyping tumoral DNA using a single biopsy [55]. Altogether, this new understanding of cancer complexity is the driving force in the development of diagnostic tests for the molecular profiling of tumors, which may guide the choice of suitable personalized therapies for each patient [52].

4. Emerging methods in HCC

The stability of potential biomarkers poses a major challenge is greater over the complications associated with the harvest and enrichment of candidate biomarkers from complex natural protein mixtures (such as blood) [46]. Immediately after blood collection (e.g., by venipuncture), proteins that present in the serum are at risk of degradation by endogenous proteases or exogenous environmental proteases, such as proteases associated with the blood clotting process, enzymes shed from blood cells or associated with bacterial contaminants [56].

During transportation and storage, there is an increasing chance for the degradation of candidate diagnostic biomarkers in the blood. When the serum and other body fluids that are collected from a multiple institutions and different locations as large repositories where samples may be shipped without freezing, the fidelity of biomarkers becomes an even more important issue [46].

The fundamental and serious physiologic barriers upsetting biomarker discovery and measurement is the extremely low abundance (concentration) of candidate markers in blood and urine. Low limits of the biomarkers are very difficult to detect by mass spectrometry and conventional immunoassays. And also in the early stage of disease, the tissue contains a small proportion of the patient's tissue volume, thus generating a low amount of biomarkers [46].

The resident proteins such as albumin and immunoglobulins are the next hindrances, which account for greater than 90% of circulating plasma proteins, as it confound and mask the isolation of rare biomarkers. When compared to the rare

biomarker, the resident proteins such as albumin exist in billion fold excess. The major problem is that the majority of low abundance biomarkers are non-covalently and endogenously associated with that resident proteins [46].

After blood or urine collection, the low abundance biomarkers are rapidly degraded by endogenous and exogenous proteinases. And also during transportation and storage of blood, candidate biomarkers are degraded which lead to serious false-positive and false-negative results [57].

Affinity bait hydrogel nanoparticles have been recently proposed in order to address these fundamental roadblocks to biomarker purification and preservation [58]. The nanoparticles contain a bait that targets classes of analytes. The nanoparticles simultaneously conduct molecular sieve chromatography and affinity chromatography, in one step, when it combined with a body fluid such as blood or urine [57]. The nanoparticles sequester all target molecules away from albumin association and completely exclude albumin [58]. It is proposed that proteins sequestered by the nanoparticles are eluted in small volumes, thus increasing their concentration, allowing analysis by a variety of techniques, such as mass spectrometry, western blotting and immunoassays [57, 58].

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