EDITORIAL

Proteomics approaches for early detection and targeted therapy of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer related mortality worldwide. HCC incidences have increased worldwide though more prevalent in Asia and Africa. Hepatitis B virus and hepatitis C virus infections are mostly responsible of increased number of HCC cases. Biomarkers can help early detection and improve treatment regimen in patients as advanced stage is chemo-refractive with limited treatment options. Potential of proteomics in finding new biomarkers for early detection has been explored more recently. Future developments in this area rely on how efficiently we manage vast amount of data generated by these techniques and speed up the clinical trials to improve patient care.

Key words: Proteomics; Cancer; Biomarker; Hepatocellular carcinoma

Core tip: Despite ongoing development in treatment for hepatocellular carcinoma (HCC), effective biomarkers for diagnosis and treatment for HCC are not available. Profiling of proteins puts proteomics on the forefront to understand promising new biomarkers and drug targets for HCC. HCC proteome database would be an important step towards identifying tumor associated proteins as potential therapeutic targets in the treatment of HCC.

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Hepatocellular carcinoma (HCC) is most common in

underdeveloped and developing countries[1,2]. Etiological agents of HCC vary in different part of world. HCC is more common in Asia due to chronic hepatitis B virus (HBV) infections whereas hepatitis C virus (HCV) infection is the major cause of HCC in western countries and Japan. Besides infection, alcoholic fatty liver disease, nonalcoholic fatty liver disease as well as nonalcoholic steatohepatitis also accounts for HCC in developing and developed countries. Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are the risk factors for HCC in China and sub-Saharan Africa. Inherited metabolic diseases, against a background of cirrhosis or without cirrhosis are associated with HCC. Smoking, estrogens, androgens, and thorium oxide (thorotrast) are also associated with HCC. Alcohol is responsible for HCC in both developing and developed countries[3]. Less common and emerging risk factors also include diabetes and obesity.

Curative surgery is not possible in HCC patients due to late diagnosis and/or advanced underlying liver cirrhosis[4]. Only limited treatment options such as resection and transplantation, radiofrequency ablation and transarterial chemoembolization with marginal clinical benefits are available for majority of patients. The cytotoxic systemic therapy options usually fail in patients with HCC due to the chemoresistance[5]. Though most commonly used drug is Doxorubicin[6], a combination of Sorafenib and Doxorubicin therapy is more beneficial in patients with advanced HCC[7]. Recent reviews summarized a list of agents for the treatment of HCC[8,9]. Despite ongoing development in treatment for HCC, effective biomarkers for diagnosis and treatment for HCC are not available.

Alpha-fetoprotein (AFP) is a biomarker, currently used for screening patients at-risk of HCC. AFP is not a good marker as many other liver diseases can also increase blood level of AFP. Furthermore, AFP is not always elevated in early stages of cancer development, when therapy is mostly effective. Other serum markers for HCC have now been identified in addition to AFP. These are, for example, an abnormal prothrombin molecule, des-γ-carboxyprothrombin, cell-surface proteoglycan, glypican-3, glycoprotein, osteopontin, golgi protein 73, microRNA-21, α-1-fucosidase, human telomerase reverse transcriptase, squamous cell carcinoma antigen, and transforming growth factor-β1. Data suggest that a combination of biomarker may be more effective[10]. More importantly, nine Food and Drug Administration-approved blood based cancer markers are used to monitor the treatment[11]. Profiling of proteins puts proteomics on the forefront to understand promising new biomarkers and drug targets for HCC.

HCC proteome database would be an important step towards identifying tumor associated proteins as potential therapeutic targets in the treatment of HCC[12]. Basic proteomic approaches including 2-dimensional electrophoresis (2DE), reversed-phase high performance liquid chromatography, size-exclusion chromatography, free-flow electrophoresis, capillary electrophoresis, ion-

exchange chromatography along with tools such as MS-based imaging of tissue biopsies, plasmon resonance technique coupled to MS, matrix assisted laser desorption/ionization-time of flight mass spectrometry, surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS), 2DE-LC-MS/MS and laser capture microdissection enable the study of cancer proteomics[13]. Most significant advancement in 2DE is two-dimensional difference gel electrophoresis (2D-DIGE), with greater sensitivity and dynamic range due to use of fluorescent cyanine dyes (Cye2, Cye3, and Cye5)[14]. Analysis of HBV related HCC tumor and non-tumor tissues using 2D-DIGE revealed increased expression of heat-shock proteins (hsp70, hsp90) and heterogeneous nuclear ribonucleoproteins (C1 and C2) as tumor biomarkers[14].

Bioinformatics is essential for proteomic analyses. The Human Proteome Organization (HUPO) has developed the standards for experimental strategies and data exchange[15]. An open basic XML (extensible markup language) representation of MS data, named mzXML, helped accelerate data management, interpretation and dissemination using different instrumentation platforms[16]. Commercial tools available to the proteomics community to analyze two-dimensional electrophoresis protein patterns include Delta2D (Decodon), BioNumerics 2D (Applied maths), Melanie (GeneBio), Imagemaster 2D (GE healthcare), Progenesis Samespots (NonLinear Dynamics), PDQuest (BioRad Laboratories), REDFIN (Ludesi), ProteinMineTM (Scimagix), and the Z3:2D-Gel image Analysis System (Compugen Limited).

Global analysis of proteins faces several challenges, for example, tertiary structure of proteins, detection of low abundance proteins and reversible modifications such as glycosylation and phosphorylation when compared to studies of genes and transcripts. Furthermore, RNA splicing can produce splice variants that are homologous but differ in function. The revolution in the field of proteomics can hopefully overcome some of these hurdles. In last forty years, only few new tests have been added in clinics. A four-way collaboration is required between the research laboratory (for developing the fundamental concept), the diagnostic lab or industry (converting the concept into a hands-on reliable tool), the clinical laboratory (assessing the tool in real life practices), and the clinicians (providing clinical specimens) for bringing a biomarker from the research lab successfully into the clinical practice[17].

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