Systems Challenges of Hepatic Carcinomas: A Review

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Hepatocellular Carcinoma (HCC) is ubiquitous in its prevalence in most of the developing countries. In the era of systems biology, multi-omics has evinced an extensive approach to define the underlying mechanism of disease progression. HCC is a multifactorial disease and the investigation of progression of liver cirrhosis becomes much extensive with cultivating omics approaches. We have performed a comprehensive review about such challenges in multi-omics approaches that are concerned to identify the immunological, genetics and epidemiological factors associated with HCC. (J CLIN EXP HEPATOL 2019;9:233–244)

The last two decades has seen a continuous surge in the incidence of Hepatic Carcinomas (HCC).¹ According to GLOBOCON report, it is estimated that 782,000 new liver cancer cases were reported worldwide in 2012 alone making it the most common malignancy in adults, the sixth most prevalent cancer in the world and second in number in cancer associated deaths.^{2,3} The sex ratio indicates that it is the fifth most common cancer in men and second most frequent cause of cancer associated deaths, whereas in women, it is the ninth most common cancer and sixth in cancer related deaths.² This clearly outlines its incidence more in men when compared to women. Higher rates of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infections in male population, high alcohol intake and obesity are likely to be reasons for this discrepancy (Figure 1).⁴ Its prevalence is more pronounced in developing countries (>80%) than in developed nations.⁵ This geographical dissimilarity is perhaps related to the difference in the global distribution of hepatotropic virus being more common in Asia and south-central Europe.⁶ Through this article, we review the challenges associated with epidemiological, immunological and genetic factors that HCC has seen in recent-past.

Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), alcohol, liver associated diseases like Non-Alcoholic Fatty

Liver Disease (NAFLD), Non-Alcoholic Steatohepatitis (NASH), exposure to aflatoxins and metabolic disorders like diabetes, obesity, and hemochromatosis are the major risk factors associated with HCC. Continuous exposure to these causes liver fibrosis eventually leading to liver cirrhosis and ultimately HCC.

IMMUNOMODULATORS AND CARCINOMAS

During 19th century, various studies indicated that there is an association between cancer and immune system which explained the innate and adaptive immune response mechanisms.⁷ As a part of this process, transformed cells are first recognized by natural killer cells (NK cells) by specific interactions with ligands on tumor cells and destroy them. These destroyed tumor cells are phagocytosed by macrophages and dendritic cells and in response secrete cytokines that activate T and B cells which, in turn activate innate immune system and eliminate cancer cells. In addition to innate immunity, adaptive immunity also eliminates tumor cells by employing activated CD4⁺ helper and CD8⁺ cytotoxic T cells, and antibodies.⁸ These findings clearly implicate the role of host immune system in cancer rejection or progression which provides the causative link between inflammation and tumor development. Tumor cells secrete cytokines and chemokines that attract various leukocytes such as neutrophils, dendritic cells, macrophages, eosinophils and mast cells which induce inflammatory responses⁹ (Figure 2). Tumorigenesis can be mediated by many factors such as genetic mutations in somatic and germline cells, life style, epigenetic, immune dysfunction, alteration in molecular pathways, environmental factors etc.¹⁰ Most environmental and other risk factors of cancers are associated with chronic inflammation and one such entity is inflammatory microenvironment which is an essential

Keywords: hepatocellular carcinoma, immunomodulators, signaling pathways, systems biology

Received: 23 February 2018; **Accepted:** 10 May 2018; **Available online:** 17 May 2018

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Abbreviations: HBV: Hepatitis B Virus; HCC: Hepatocellular Carcinoma; HCV: Hepatitis C Virus; NAFLD: Non-Alcoholic Fatty Liver Disease

<https://doi.org/10.1016/j.jceh.2018.05.002>

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Figure 1 Different stages of HCC. Figure depicting various stages of HCC. Stage 1. Liver Fibrosis: Formation of scar tissue within the liver; Stage 2. Liver Cirrhosis: Extensive scarring blocks the blood flow through the liver deteriorating its function; Stage 3. Liver Cancer: Formation of malignant tumors.

factor for tumor development as well as modulation of host immunity. In addition, many bacterial and viral infections were also shown to induce inflammation and increase the risk of cancer. Along with inflammation the

immunomodulation of the host can encourage or combat the carcinogenesis and spread of tumors.¹¹ For instance, chronic inflammation plays an important role in the development of HCC.¹²

Figure 2 Figure depicting various cells of immune system which gets modulated into transformed cells and helps in progression of HCC.

The changes in number or function of various immune cells, cytokine levels, expression of inhibitory receptors or their ligands contributes to the development and progression of HCC.¹³

CYTOKINES AND CHEMOKINES AS FACTORS

One of the most important components of the immune system is cytokines, a secreted or membrane-bound protein released in response to cellular stresses such as inflammation, infection and carcinogen-mediated injury. These proteins regulate growth, differentiation, and activation of immune cells.¹⁴ The cytokines can be divided into two classes – pro-inflammatory and anti-inflammatory and many of which are pleiotropic.¹⁴ Cytokines secreted by

CD4+ Th cells are defined as Th1 and Th2 cells comprising of Interleukins (ILs). Proinflammatory response (e.g., IL-1 α , IL-1 β , IL-2, IL-12p35, IL-12p40, IL-15, and non-ILs, e.g., TNF- α and IFN- γ) is exerted by Th1 cytokines while the Th2 cytokines (e.g., IL-4, IL-8, IL-10, and IL-5) induce anti-inflammatory responses.¹⁵ Hepatocytes express receptors for many cytokines and thus make the liver susceptible for cytokine-mediated responses. There is an evidence about the role of cytokines in the progression of HCC.¹⁶ In response to infections and inflammation caused by hepatitis viruses (HBV and HCV), the inflammatory cells secrete cytokines and chemokines, which contribute to the liver damage and progression of HCC. The cytokines that were shown to be involved in the hepatocarcinogenesis are IL-1 β , IL-2, IL-1 β , IFN- γ , TNF- α , IL-4, IL-10, IL-6, and TGF β . Studies have revealed that Th1 cytokines namely, TNF,

IFN- γ , IL-1 α , and IL-1 β were downregulated while the levels of cytokines secreted by Th2 cells, namely IL-4 and IL-5, IL-8, and IL-10 were shown to be elevated in liver associated with metastatic HCC compared to normal liver.¹⁷ This indicates a Th1-Th2 cytokine shift in the tumor microenvironment within the hepatic tissues from HCC patients.¹⁸ Apart from a pro-inflammatory cytokine and contributing to tumor growth, IL-1 β has other important roles in the development of gastric cancer and hepatitis-C induced HCC.¹⁹ In addition, levels of IL-1 β have shown to induce the production of other cytokines, IL-2, IL-6, and TNF- α in HCC patients.¹³ Interferons are well known to be involved in immunomodulation with researchers finding decreased levels of IFN- α , IFN- β , and IFN- γ in serum samples of HCC patients.²⁰ Apart from the above-mentioned facts, the mechanism behind the HCC has been associated with several molecular signaling. They are of importance from a therapeutic point of view as targeting the pathways can help reduce or prevent HCC.²¹ Pathways such as Wnt- β catenin, EGFR-Ras-MAPKK, c-MET, IGF, Akt/mTOR, VEGF, PDGFR, and others have been known to contribute toward development and progression of HCC and hence, efforts are made to develop therapeutic targets by aiming the pathways.²² For instance, c-MET/HGF signaling is dysregulated in HCC.²³ Drug targets are being developed by producing antibodies against c-MET/HGF receptor or producing c-MET specific inhibitors.²⁴ Likewise, in Hedgehog signaling, the expression of smoothened (SMO, a transmembrane receptor) was found to be upregulated in HCC tumors when compared to normal. By using a steroid alkaloid, cyclopamine, it is possible to inhibit Hedgehog signaling by binding and blocking SMO, which, in turn, increases apoptosis and decreases cell proliferation.²⁵

It is interesting to note that chemokines play a dual role in the inflammation of HCC. Either, they act as a mediator for inflammation by getting incited by different cytokines, or they instigate the cytokine secretion in the HCC

microenvironment. The fact that they are of great interest for researchers lies in their role in luring the immune cells toward the tumor environment and various studies have confirmed the presence of chemokines in the cancerous cell lines.²⁶ They have been shown to report to impact the chemotaxis effect on HCC cells. One such instance is found during the study where CXCL12 binds to CXCR4 to activate Matrix Metalloprotease-9 (MMP-9) and Matrix Metalloprotease-2 (MMP-2), which results in migration and invasion of HCC cells.^{27,28}

In addition, there are various cells of the host immune system that are directly involved in immunosuppression. These include CD4⁺ Cytotoxic T lymphocytes (CD4⁺ CTLs), CD4⁺CD25⁺ Tregs (regulatory T cells), Myeloid-derived Suppressor Cells (MDSCs), Natural Killer (NK) cells, and dendritic cells. CD4⁺ CTLs are subsets of CD4⁺ T cells, which play an important role in pathogenesis of HCC (Figure 3). Fu et al.²⁹ reported a decreased level of CD4⁺ CTLs in liver tumor tissue from patients with HBV-infected HCC. This decreased level indicate the immunosuppressive state of tumor microenvironment.³⁰ They also demonstrated an inverse relationship between Tregs and CD4⁺ CTLs. Unlike the CTLs, an increased number of CD4⁺CD25⁺ Tregs were found in the blood of the patients with HCC.³¹ Next in line are the MDSCs resulting from the differentiation of the myeloid cells mediated by the cancerous cells. Studies have revealed that the population of MDSCs increase in the peripheral blood of patients with HCC and a correlation was indicated with stages in the cancer. MDSCs could also suppress the T-cell proliferation and IFN- γ secretion confirming its suppressive effect.³² NK cells are the first line of defense against cancers and infections. Research on NK cells have indicated a reduction in frequency of NK cell subsets in HCC patients compared to healthy and the number of NK cells found in the cancer cells has a positive correlation with survival rates of the patients.^{33,34} Dendritic cells are Antigen Presenting Cells (APCs) that function in homeostasis

Figure 3 Possible mechanisms by which CD4⁺ Cytotoxic T lymphocytes, CD4⁺ CD25⁺ Treg cells, NK cells, DCs and Th17 cells exert protective role in immune system.

Table 1 Genes Known to Regulate HCC.

Signaling pathway	Biological effect	Genes mutated in HCC	References
Growth factor signaling	Cell proliferation and survival	TGF α , EGF, VEGF and EGFR	Llovet and Bruix, 2008 ²² Breuhahn et al., 2006 ⁵⁴ Villanueva et al., 2007 ³⁹
IGF signaling	Regulation of growth and survival of hepatocytes, liver regeneration after hepatectomy. Cell proliferation and apoptosis. Major role during embryonic development and differentiation.	IRS-1, IRS-2, IGF II	Branda et al., 2006 ⁵⁵ Llovet et al., 2008 ²² Bruix, 2004 ³⁸ Breuhahn et al., 2006 ⁵⁴
HGF/c-MET signaling	Hepatocyte regeneration followed by injury	HGF, MET	Breuhahn et al., 2006 ⁵⁴
Wnt/ β -catenin signaling	Regulate fundamental processes of tissue formation during embryogenesis. Cell proliferation antiapoptosis, invasion and angiogenesis	β -Catenin, APC, Axin-1, Axin-2, FZD-7	de La Coste et al., 1998 ⁵⁶ Ishizaki et al., 2004 ⁵⁷ Merle et al., 2004 ⁵⁸ Wodarz and Nusse, 1998 ⁵⁹
PI3K/Akt/mTOR signaling	Cell growth and survival	PI3K, PTEN	Villanueva et al., 2007 ³⁹ Tanaka et al., 2006 ⁶⁰ Lee et al., 2005 ⁶¹ Sabatini et al., 2006 ⁶²

and are most importantly considered in immunotherapy as they have increased antitumor activity. Its role in restraining immunoregulatory mechanisms and promotion of effector cells against tumor indicates toward the tumor eradication from cancer patients.³⁵ In relation to HCC, it has been observed that decreased numbers of DCs are found in the blood of the patients.³⁶ DCs are also being explored as a role toward vaccination and like in any other clinical trials, the safety and efficacy are a major concern and hence this area seeks attention. Recent studies showed anti-tumor activity in presence of DC vaccines in patients with advanced HCC and liver cirrhosis, except for one patient who showed a clinical response.³⁷

SEVERAL GENES ARE KNOWN TO REGULATE HCC

Several events like mutations, genomic or epigenetic modifications and transformed or deregulated signaling pathways are the major molecular abnormalities underpinning HCC.²² Large number of genes with increased or reduced expressions are found to be associated with conditions leading to HCC (shown in Table 1). Some of the pathways regulated by these genes include cell cycle regulation (*p53*, *p16*), cell proliferation and differentiation (β -catenin, *c-myc*, *APC*, *E-cadherin*), apoptosis (*bcl2*), angiogenesis (*VEGFR-2*, *Angiopoietin-2*), metastasis (*MMP4*, *MMP9*, *Topoisomerase*, *Rb*, *Cyclin D1*, *Osteopontin*) and other growth factor signaling components (*IGF-II*, *EGFR*, *TGF*, *HGF/c-MET*, *K-RAS*, *PIK3CA*, *PTEN*).^{38,39} Among these, *p53*, *PIK3CA*, and β -catenin are the most commonly mutated genes in HCC.³⁹ Kancherla et al. identified V157 and R249, two distinctive

mutation hotspots in HCC which are extremely rare in other types of cancers. Their study suggested that somatic mutations harboring TP53 displaying necrotic areas were very frequent in HCC. Other significant observations were four TP53-mutant subsets including CTNNB1 mutations, 1q amplifications or 8q24 amplifications co-occurred with TP53 mutations. Further, a mutational signature 12, prevalent in HCC with wild-type or missense TP53 mutations was also found.⁴⁰ Mao et al. also reported R249S mutation in TP53 at a frequency of 7.7% in HCC patients.⁴¹ Andrade et al. disclosed that a patient diagnosed with fibrolamellar hepatocellular carcinoma, a rare subtype of HCC carried a missense germline pathogenic variant 467G>A in exon 5, a silent germline variant 582T>C in exon 6 both in heterozygous condition and further analysis revealed an additional somatic mutation 461G>A in TP53 gene. All these reports suggest potential role of mutations in TP53 gene in progression of HCC. In extension to mutations, TP53 expression is altered by various other factors such as chromosomal abnormalities and viral infections either directly or indirectly.⁴² Structural changes in chromosomes resulting in loss or gain of alleles have been detected in few chromosomes in more than 30% of HCCs. While tumor suppressor genes like *p53*, *Rb* and other CDKN2A fall in these regularly deleted chromosomal regions⁴³⁻⁴⁵ epigenetic silencing of cancer related genes by unusual DNA methylation pattern also have been reported in HCC while *p16* and *E-cadherin*, *COX2*, *ASC*, *DLC1* are the genes mostly targeted by hypermethylation.⁴⁶

Carcinogenesis of HBV-associated HCC is due to the integration of viral DNA into the host genome causing transactivation of oncogenes, chromosomal rearrangement

and genomic instability in host hepatocytes.⁴⁷ HBV genome integration results in the loss of host DNA sequences (also known as host DNA microdeletions) that include several cancer related genes. Two tumor suppressor genes; adenomatous polyposis coli-like (APCL) and O6-methylguanine DNA methyltransferase (MGMT) and five apoptosis related genes; telomerase reverse transcriptase (TERT), thyroid hormone associated protein 150 (TRAP 150 α), scavenger receptor class A member 3 (SCARA3), mitogen associated protein kinase 1 (MAPK1) and BCL2-like 2 (BCL2L2) are the frequently integrated sites for HBV. Platelet derived growth factor receptor- β (PDGFR β), genes involved in calcium signaling and 60S ribosomal protein formation are the other genes that are regularly targeted.^{46,48} HBx, one of the proteins encoded by HBV, owing to its transcriptional activation ability, activates the expression of growth control genes (Ras, Raf, MAPK, ERK, JNK, SRC tyrosine kinases etc.) and inactivates p53, as a result enhancing cell proliferation and survival.^{46,49,50}

The HCV single stranded positive-sense RNA genome encodes structural (core, E1, E2, and p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins.^{46,51} NS5A has been shown to interfere with MAPK signaling pathway thereby modifying cell division⁵¹ p21/waf1 (cyclin dependent kinase inhibitor) gene promotes apoptosis and is upregulated by p53 resulting in G1 arrest. NS5A down-regulates p21/waf1 promoter activity by blocking the access of p53 to the p21/waf1 promoter thus altering p53 regulated cellular pathways.⁵²

Genes associated with major signaling pathways are also altered in HCC. Many of these critical pathways connected to the pathogenesis of HCC include receptor tyrosine kinase pathways, the Wnt/ β -catenin signaling pathway, IGF signaling, c-met signaling, PI3 kinase/AKT/mTOR pathway and the ubiquitin/proteasome degradation pathways.⁵³

ROLE OF MIRNAS IN HCC

Small non-coding RNAs are categorized into microRNAs (miRNAs) and Piwi-Interacting RNA (piRNAs), which acts as gene regulators in both plants and animals. The miRNAs are expressed differentially in different malignancies, thus they either function as oncogenes or tumor-suppressor genes. Furthermore, circulating miRNAs may function as biomarkers in HCC, as they show various pathophysiological features of HCC.⁶³ In addition miRNAs are also related to Okuda grading, tumor size, and multiple hepatic focal lesions in order to work as a prognostic marker for HCC.⁶⁴ A list of miRNA's, which covered seven miRNAs by Zhou et al. served as a signature to indicate differences between HCC patients and cirrhosis, chronic infection with HBV, and healthy subjects.⁶⁵ Recent bioinformatics databases *viz.* TarBase, miRSystem, TargetScan,

miRanda, Diana have paved out a useful way to hunt for miRNA targets thus assisting in finding biological processes, molecular functions, cellular processes, and important pathways associated with HCC.⁶⁶ miRNAs are also known to be involved to target different components in inflammatory pathways such as NF- κ B and STAT3 signaling pathways, which is involved in malignancy, as HCC is developed from severe liver inflammation.⁶⁷ Furthermore, miRNAs, in PI3K/MAPK pathways also promotes cell proliferation, migration, and invasion, induces perturbation in the JAK/STAT pathway, and plays a role in TP53 and WNT/ β -catenin pathways. In addition, miRNAs regulate their target gene expression, involved in biological processes such as apoptosis, cell cycle regulation, angiogenesis, and metastasis and the miRNAs targeted genes mainly acts as enzyme binding, dehydrogenase, and glutamate transmembrane transporter and so on.^{68,69} HCC includes several secondary conditions cirrhosis, viral infection, fibrosis, and inflammation, and miRNA biomarkers being not specific, could have been triggered by any of the above mentioned conditions, which is a caveat in the assessment of using miRNAs in HCC. As multiple miRNAs target genes, it would be reasonable to select a panel of deregulated miRNAs that can be used as a diagnostic tool in HCC.⁷⁰

GENOMIC VARIANTS ASSOCIATED WITH HCC: SYSTEMS BIOLOGY CHALLENGES

In the recent-past, mutations specific to cellular components and genes associated with HCC, microsatellite instability and epigenetic changes have been documented.⁷¹ Knowledge of functional effects associated with these mutations are useful to understand the variome (genetic components associated with the variation of entire organism), transcription, metabolomics and metabonomics. As it is possible to discern these structures in the near future, there is a need to understand and develop custom-made therapeutic targets for HCC which will allow patients toward improved survivability. This will also allow researchers to define predictive biomarkers.⁷²

Biomarkers are the indicators present in the body fluids that indicates the status of the disease. Some of the serum biomarkers used in diagnosis and screening of HCC are presented here:

Alpha fetoprotein (AFP): AFP is the most common biomarker in serum used in the diagnosis of HCC. It is a glycoprotein that is produced during pregnancy by embryonic endodermal cells of the visceral yolk sac during early stages of liver development under physiological conditions. AFP evaluation can occur in hepatocyte regeneration, during hepatocarcinogenesis and is useful for screening and diagnosis of HCC and as a marker for detecting tumor progression in AFP positive HCC

patients. But, AFP testing lacks sensitivity and specificity for effective surveillance with limited diagnostic accuracy in detecting small HCC and mainly restricted to those areas endemic with HBV.⁷³⁻⁷⁶

AFP-L3: It is an isoform of AFP. It is used as an early biomarker of HCC when the tumor size is around 2 cm. As the size of tumor increases, the sensitivity of this marker is increased.^{73,74,76}

APO-J: APO-J/Clusterin is a glycoprotein with seven glycosylated sites. It is more sensitive and specific when compared to AFP. APO-J expression was shown to be significantly decreased in HCC patients compared to healthy controls and can be used as an independent marker of HCC. It can also be used as a prognostic marker and can also monitor HCC progression and metastasis.⁷³

DKK-1 Dickkopf-1: DKK is of high importance in cases where AFP biomarker fails to diagnose HCC. Especially, it can diagnose HCC in very early stages and effective for HCC patients undergoing liver transplantation (LT) in predicting the prognosis of the disease.⁷³

Human carbonyl reductase-2 (HCR-2): HCR-2 is expressed in human liver and kidneys. During oxidative stress, the reactive oxygen species and alpha dicarbonyl released are detoxified by this enzyme. The expression of HCR2 is significantly decreased in the HCC when compared to normal cells.^{73,75}

Wnt: Wnt-1 protein is a prognostic biomarker of HBV- and HCV-related HCC after surgery. GPC3 molecule promotes the growth of HCC by stimulating Wnt signaling.⁷³

Angiopoietin-1/2: Angiopoietin-2 expression was shown to be upregulated in HCC and cirrhosis, and this could be used as a marker to detect advanced pathological invasiveness and overall survival of HCC patients.⁷³

NOTCH: Activation of NOTCH plays a prominent role in HBV-mediated HCC. Thus, NOTCH1 is one of the possible therapeutic targets for the treatment of HBx-associated HCC. NOTCH1 and NOTCH4 were proven to be potential biomarkers for the poor prognosis of HCC by recent studies.⁷³

Oncostatin M: OSM is a cytokine secreted from the hematopoietic cells in early stages and induces the differentiation of hepatocytes by modulating HNF4 alpha. It regulates cytokine production, such as IL-6, GM-CSF, and G-CSF and was shown to be elevated in HCC.⁷³

Alpha-1 antitrypsin: Alpha-1 antitrypsin is a member of the SERPINA1 family of proteins, controlled by IL-6, TNF α , and IL-1. Elevated levels of A1AT was shown to be associated with HCC. Alterations in the status of A1AT glycosylation was observed during HCC. Compared to cirrhosis, it is significantly elevated in HCC and could also be used as a differentiation marker.⁷³

WFA+ M2BP: An assay using Wisteria floribunda agglutinin-positive human mac2-binding protein is used for assessing liver fibrosis and this can be used as an independent risk factor biomarker for HCC development.

Interestingly, WFA+ M2BP can predict HCC in HCV patients who respond well to the treatment.⁷³

Lymphotoxin beta receptor: Lymphotoxin beta receptor is a cytokine and a member of tumor necrosis factor family, which is involved in controlling the development of lymphoid organs. It is widely expressed with sustained oncogene activity in HCC and hence can be used as a marker.⁷³

Chromogranin A (CgA): CgA is a glycoprotein secreted by neuroendocrine cells under physiological conditions. Its high concentrations in serum samples of HCC patients, suggests that it might be a useful biomarker in monitoring cirrhosis patients for the early detection of HCC. Since its levels were shown to be increased in both HCC and cirrhotic patients, it presents a low diagnostic specificity. CgA can be used in monitoring the efficacy of HCC treatment.⁷⁵

Nerve Growth Factor: Nerve Growth Factor (NGF) is a member of neurotrophin family that plays a vital role in differentiation, survival, and preservation of peripheral and central nervous systems. In addition it is also involved in tumor growth, invasion and metastasis. There are two receptors of NGF: high-affinity trkANGF and low-affinity p75NTR. Expression of NGF and trkANGF increases significantly during HCC.^{73,75}

Serum Anti-p53: The p53 gene is a tumor-suppressor gene encoding a nuclear phosphoprotein that inhibits cellular proliferation and transformation. Mutations in p53 gene occur at the advanced stages of HCC. The abnormalities in the gene relate to the prognosis and survival of HCC patients. Anti-p53 antibodies in serum of HCC patients could be a potential diagnostic marker.⁷⁵

Midkine: Midkine is a heparin-binding growth factor. It is expressed during wound healing, tumorigenesis, and inflammation. Midkine expression markedly rises during early development of HCC but not in advanced stages of HCC. Therefore, it can be used as biomarker for early detection of HCC.^{73,74}

Golgi protein 73 (GP73): GP73 is a transmembrane protein of Golgi complex whose expression was shown to increase at significant levels in patients with HCC than with cirrhosis. Therefore, GP73 is appropriate for diagnosing patients with small and early-stage HCC.^{74,75}

Des- γ -carboxy prothrombin: It is an important biomarker for large size HCC, especially in cases where AFP fails to diagnose HCC, des- γ -carboxy prothrombin can detect HCC. It is a prothrombin protein of VEGF family expressed during vitamin K deficiency/antagonist-II (PIVKA-II). DCP is involved in angiogenesis in tumors by increasing the expression of angiogenic factors such as VEGF, EGF-R, and MMP-2 resulting in the proliferation and migration of human vascular endothelial. Upregulation of DCP can be correlated with the degree of malignancy in HCC. Various studies reported that DCP is a superior diagnostic biomarker to both total AFP and

AFP-L3 particularly in differentiating between HCC and non-malignant hepatic cirrhosis with a high sensitivity and specificity. Higher DCP levels in HCC patients are associated with a poorer prognosis.^{73–76}

α -1-Fucosidase: α -1-Fucosidase is a lysosomal enzyme that hydrolyzes fucose glucosidic bonds present in glycoproteins and glycolipids. Its expression increases during chronic hepatitis, cirrhosis, and HCC. It is one of the early HCC biomarkers and studies showed that it is raised preceding six months of development of HCC. AFU measurement is useful in association with AFP in the early diagnosis of HCC.^{73,75}

Hepatocyte growth factor: HGF is a cytokine produced by nonparenchymal Ito cells in the liver. HGF levels increases in hepatic regeneration, chronic hepatitis, cirrhosis, and HCC. It acts as a prognostic biomarker and can predict the early tumor recurrence and metastasis. The increase of HGF serum levels in cirrhotic patients is an indicator of HCC development. Pre-operative high HGF levels are related to the development of post-operative complications, such as liver failure. Moreover, elevated HGF serum levels, after surgery, can predict early tumor recurrence and metastasis.^{73,75}

Cytokeratin 19: Cytokeratin 19 (CK19) is a novel HCC biomarker that has been consistently associated with poor clinical prognosis in patients. The simultaneous detection of CK19 and GPC3 expression in HCC patients was shown to be a predictive indicator of higher risks of cancer invasion and metastasis, as well as worse treatment outcome with better diagnostic sensitivity.⁷⁴

Transforming growth factor- β : TGF- β plays an important role in the control of cellular proliferation and differentiation in HCC cells. Serum TGF- β levels are raised in HCC patients and can be used as a biomarker as well as a therapeutic target for the treatment of HCC.^{73,75}

Embryonic Liver Fodrin (ELF): Previous studies suggested decreased expression of ELF in HCC tissues. Reports also indicated a significant negative correlation between ELF and TGF- β 1. Patients with high TGF- β 1 expression or/and low ELF expression appeared to have a poor postoperative disease-free survival and overall survival. Furthermore, the predictive range of ELF combined with TGF- β 1 was more sensitive than that of either one alone.⁷⁵

Vascular endothelial growth factor: VEGF is a glycosylated cytokine that exerts functions as a mitogen and promotes vascular permeability, angiogenesis, vasculogenesis, and endothelial cell growth-reduced survival. Tumor environment promotes VEGF expression and initiates VEGF signaling thus promoting angiogenesis, proliferation and metastasis. The development of solid tumors is strictly correlated with angiogenesis. VEGF levels predict HCC recurrence, and it is a substantial biomarker for the survival of HCC patients. VEGF levels are higher in HCC

patients especially in advanced HCC compared to early HCC.^{73,75}

Epidermal growth factor: Epidermal growth factor receptor (EGFR) signaling is vital for all the stages of hepatic injury from inflammation to HCC development. One of the forms of EGFR, ErbB3, was detected in the serum of HCC patients during the early stages of HCC development. There were studies reporting that HBV HBx protein downregulates the EGFR expression by inducing miRNA-7 in HCC cells.⁷³

Annexin A2: Annexin A2 is a calcium-dependent, phospholipid-binding protein commonly found in the cell surface. It is involved in cell mobility and protein interaction with the actin cytoskeleton, as well as endocytosis. Due to these roles, Annexin A2 has also been implicated in the development and metastasis of HCC. Overexpression of Annexin A2 was revealed to be an indicator of HCC tumor malignancy in patients and showed to be inversely correlated with their survival rates. Annexin A2 also demonstrated higher sensitivity and specificity than AFP and might serve as a serologic candidate for diagnosing and determining the prognosis of early-stage HCC patients.⁷⁴

P-aPKC-i, E-Cadherin, b-Catenin: P-aPKC-i, E-cadherin, and b-catenin play an important role in tight-junction formation among tumor cells. P-aPKC-i is a member of the family of serine-threonine kinases (PKC) that play an important role in cellular proliferation and differentiation. In normal liver tissue, it is localized at the apical membrane, while in HCC it is localized at the basal membrane and in cytoplasm. Probably, the high expression of aPKC-i causes the loss of cell polarity and cellular junction, leading to metastasis. E-cadherin is a transmembrane glycoprotein, connected with its intracellular domain, through b-catenin and other catenins, to the acting cytoskeleton. The reduced expression of E-cadherin is associated with inhibition of the formation of a tight junction among tumoral cells and is correlated to development of metastasis and a poor tumoral differentiation. B-catenin overexpression in HCC tissues seems to be involved in activation of the WNT signaling pathway and in expression of c-myc, cyclin D, VEGF, and other genes related to cell proliferation.⁷⁵

Squamous cell carcinoma antigen: Squamous Cell Carcinoma Antigen (SCCA) belongs to family of serpins, serine protease inhibitors and is present in squamous epithelium. SCCA as a supplementary diagnostic marker for HCC in addition to AFP.^{74,75}

Osteopontin: Osteopontin (OPN) is a secreted and highly phosphorylated Extracellular Matrix (ECM) protein functionally implicated in a diverse range of biological processes, such as bone remodeling, chemotaxis, ECM degradation, and inflammation. OPN is upregulated in various types of malignancies. The OPN has an ability to provide better discrimination for HCC from cirrhosis

compared to AFP and its upregulation in plasma could be detected well in advance. As OPN is an extracellular protein with angiogenesis potential, it can be used as a therapeutic target for inhibiting HCC metastasis. The only limitation associated with it is that it should best be used in combination with one or more HCC-specific biomarkers for overall reliability and accuracy of the screening approach.⁷⁴

Glypican-3: Glypican-3 (GPC3) is a member of glypican family and consists of various glycosylphosphatidylinositol-anchored cell surface heparin-sulfate proteoglycans. It plays an important role in cell proliferation and tumor suppression. GPC3 was upregulated in HCC tissues obtained from patients. The soluble fraction of GPC3 located at N-terminal was reported to use as a complementary serologic biomarker with better diagnostic performance than AFP due to its ability to accurately distinguish between patients with small, well-differentiated HCC tumors and those with cirrhosis. Recent studies demonstrated the therapeutic potential of a GPC3 peptide-based vaccine against advanced HCC. It remains a biomarker of interest because of its more biologically plausible link to HCC, although poor sensitivity limits its application in screening.^{74–76}

In a remarkable study, Qing-Hai Ye et al.⁷⁷ applied cDNA microarray-based gene expression profiling to investigate the global changes associated with HCC metastasis. When they tried to differentiate between primary tumors from the similar intra-hepatic metastatic lesions based on such profiling, no basis of distinction could be found. But the primary HCC with and without metastasis were distinguishable from each other. Additionally, they also found that osteopontin, a secreted phosphoprotein could be a responsible factor for HCC metastasis and this could be used both as a molecular marker for detection of HCC with a metastatic potential and also as a potential therapeutic target for metastatic HCC.

It has been suggested for long time that there is a significant difference or variability between the diversity of peptides released by tumor cells and the normal somatic cells. These patterns in the diversity and population counts of peptides could be interpreted as specific “fingerprints” and these could be isolated from blood samples and analyzed by mass spectrometry (MS). Comparison of the combinations of these multiple peptide signals with control or normal cells could reveal cancer specific changes. Chauhan et al.,⁷⁸ proposed several tissue markers for their prediction of risk of HCC development. In practice, however, α -1-Fetoprotein (AFP) is the only serum marker routinely used for diagnosing HCC. But the test is not much effective for early HCC as reported by Mann, et al.,⁷⁹ Daniele, et al.,⁸⁰ Bruix, et al.³⁸ and others. So there is a pressing need to find more effective biomarkers for prediction of HCC in developed as well as early stages of the disease. The exhaustive study by Camaggi

et al.⁸¹ is an exemplary work in detection of early and different other stages of HCC. They used matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) proteomic signature of peptides bound to serum albumin (coined by them as ‘albuminome’) from 45 study subjects having representing cases of hepatitis C virus (HCV) related cirrhosis; small, unifocal HCCs and also advanced HCCs. To ensure adequate quality control and normalize sampling errors, they extracted 4–8 samples from each subject and thus a total of 522 subject samples and 299 quality control spectra were analyzed. The data analyzed using random forest algorithm was used for classification.

A major objective of this work was to identify proteomic signatures classifying different stages of chronic liver disease progression toward HCC and subsequent vascular invasiveness. They focused on a specific “subproteome”, the subset of low molecular weight (LMW) proteins or peptides bound to human serum albumin (HSA), hereafter referred to as the “albuminome”. Most of the low molecular weight peptides are found to form complexes with serum proteins with high abundance like HAS.⁸² These bound molecules thus remain in circulation for a long time and are better candidates for detection.

Thus, the challenges remain in implementation of classification algorithms for thorough prediction of such genes associated with diseases. The validity of the models vary and differ with sensitivity, specificity and accuracy differing for automated classification. As gene identification predicts structural annotation there should be a confidence value for each predicted gene. In addition, lack of detailed descriptions of software and algorithms by authors is another challenge. This challenge can be answered by comparative genomics approaches.⁸³ At present the success of correct gene prediction is measured in terms of degree to which they predict correct amino acid sequence for the given gene segment.⁸⁴

Given the paradigm that the HCC plays a role with onset of infection and changes in immune response, the challenge here is to prioritize SNPs and variants associated with the disease across the given population. There are wide number of Genome Wide Association Studies (GWAS) across cancers, but the ones specific to Gene X Environment (GXE) would be of primordial interest to see if there are genes expressed in coherent way. There are indeed pattern recognition and prioritization models which can be employed⁸⁵ not just for inherited disorders but also for cancers such as this. There is a strong case and hope for such functional studies in HCC in years to come.

CHALLENGES AND PERSPECTIVES

Over the past decade, “omics” technologies have prompted in the increase of putative cancer biomarkers. Nonetheless, before the results can be instigated in the management of cancer patients, thorough validation such

as issues of sensitivity, specificity, reproducibility, and accuracy need to be addressed⁸⁶ On the other hand, the validation of biomarker has a complex interaction with known oncogenes or oncoproteins that has conventional further links with molecular pathways implicated in malignant transformation.⁸⁷ The foremost challenge is to bring the best results from the omics research into clinical use as accurate and reliable standardized tests that integrate into the clinical work-up.⁸⁸ Every omics approach has its strengths and drawbacks. The addition of various omics data and their functional elucidation in conjunction with clinical results is another challenge. Even as omics' database offers boundless potentials for designing new therapeutic agents for cancer, they help us to understand, diagnose, treat, and monitor of cancer in the near future.⁸⁹

On the other hand, due to efficient technology, liver cancer has emerged from a deadly to a treatable disease and considerable advancements in liver cancer management have been made. The exact molecular mechanism of targeted therapies (e.g., sorafenib, erlotinib) in HCC is still unknown.⁹⁰ Further, pharmacogenomics analysis which combines available targeted therapies and unravel the mechanism of drug resistance.⁹¹

Next generation technologies such as deep sequencing are promising tools to further increase our understanding and to identify new molecular targets.⁹² Several studies have employed deep sequencing approaches and progression of genomic variation in identifying novel mutation. One of the major challenges is the interpretation of data in a meaningful context. Another major obstacle is the data integration due to lack of bioinformatics strategies.

However, although complex interactions between the diverse biological levels and different signaling pathways activated during hepatocarcinogenesis will lead to the novel strategies for the diagnosis and treatment of HCC.⁹³ Therefore, expertise and knowledge from clinicians, biologists and bioinformaticians will be crucial to achieve this ambitious goal.

With the disease characterized by multiple risk factors associated with several biomarkers, efforts have led to understanding these biomarkers in relation to diagnoses or prognosis of the diseases.⁹⁴ The challenge here is not only to develop cost effective biomarkers in combination with diagnosis but also peruse them for therapeutics.

CONCLUSION

Hepatocellular carcinoma is one of the major cancer killers and a consequence of multiple risk factors. In this review, we focused on immunological and genetic factors that appear to be associated with hepatocarcinogenesis. We made special emphasis on the impact of omics technologies in identifying predictive biomarkers for diagnosis besides serving as therapeutic targets for the treatment of

this disease. Combining these omics studies with machine learning approaches would enhance our understanding of HCC at systems level and help us to develop effective therapies for HCC. Developing predictive models using machine learning algorithms would also help us identify the patients at high risk of HCC development, as a part of predictive medicine. Thus, keeping in view of the recent developments in omics technologies and personalized medicine, improving the performance of learning algorithms, accuracy of the model, and the model validation are the future challenges that has to be answered.

AUTHORS' CONTRIBUTION

Dhatri Madduru: Immunomodulators and Carcinomas, Cytokines and Chemokines, Biomarkers; Johny Ijaq: Introduction, Genes involved in HCC, figures, table and conclusion; Sujata Dhar: Immunomodulators and Carcinomas, Cytokines and Chemokines, role of miRNAs; Saumyadip Sarkar: writing abstract; Partha Sarathi Das: Systems Biology; Silvia Vasquez: Conceptualization, formal analysis; Naresh: Challenges and Prospective, Future perspectives and Prashanth Suravajhala: conceptualization, formal analysis, proofreading the manuscript. All authors read and approved the manuscript before the final submission.

CONFLICTS OF INTEREST

The authors have none to declare.

REFERENCES

1. Bodzin AS, Busuttil RW. Hepatocellular carcinoma: advances in diagnosis, management, and long term outcome. *World J Hepatol.* 2015;7(9):1157–1167.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN. *Int J Cancer.* 2015;136(5):E359–E386.
3. Fares N, Péron JM. Epidemiology, natural history, and risk factors of hepatocellular carcinoma. *Rev Prat.* 2013;63:216–217.
4. Shariff MI, Cox IJ, Gomaa AI, Khan SA, Gedroyc W, Taylor-Robinson SD. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and therapeutics. *Expert Rev Gastroenterol Hepatol.* 2009;3(4):353–367.
5. Kim DY, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. *Liver Cancer.* 2012;1:2–14.
6. Wang CH, Wey KC, Mo LR, Chang KK, Lin RC, Kuo JJ. Current trends and recent advances in diagnosis, therapy, and prevention of hepatocellular carcinoma. *Asian Pac J Cancer Prev.* 2015;16(9):3595–3604.
7. Dziveno OK, O'Donnell-Tormey J. *Cancer and the Immune System: The Vital Connection.* New York: Cancer Research Institute; 2003.
8. Finn OJ. Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. *Ann Oncol.* 2012;23(8):viii6–viii9.
9. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420(6917):860–867.

10. Sloan FA, Gelband H. *Cancer Causes and Risk Factors and the Elements of Cancer Control. Cancer Control Opportunities in Low- and Middle-Income Countries*. Washington, DC: National Institute of Medicine; 2007.
11. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140(6):883–899.
12. Zamarron BF, Chen W. Dual roles of immune cells and their factors in cancer development and progression. *Int J Biol Sci*. 2011;7(5):651–658.
13. Sachdeva M, Chawla YK, Arora SK. Immunology of hepatocellular carcinoma. *World J Hepatol*. 2015;7(17):2080–2090.
14. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol*. 2012;12(3):180–190.
15. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. *Nat Rev Cancer*. 2008;8(11):887–899.
16. Wu H, Li N, Jin R, et al. Cytokine levels contribute to the pathogenesis of minimal hepatic encephalopathy in patients with hepatocellular carcinoma via STAT3 activation. *Sci Rep*. 2016;6(18528):1–9.
17. Budhu A, Wang XW. The role of cytokines in hepatocellular carcinoma. *J Leukoc Biol*. 2006;80(6):1197–1213.
18. Qin LX. Inflammatory immune responses in tumor microenvironment and metastasis of hepatocellular carcinoma. *Cancer Microenviron*. 2012;5(3):203–209.
19. Tanaka Y, Furuta T, Suzuki S, et al. Impact of interleukin-1 β genetic polymorphisms on the development of hepatitis C virus-related hepatocellular carcinoma in Japan. *J Infect Dis*. 2003;187(11):1822–1825.
20. Hong YP, Li ZD, Prasoon P, Zhang Q. Immunotherapy for hepatocellular carcinoma: from basic research to clinical use. *World J Hepatol*. 2015;7:980–992.
21. Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene*. 2010;29(36):4989–5005.
22. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology*. 2008;48(4):1312–1327.
23. Takami T, Kaposi-Novak P, Uchida K, et al. Loss of hepatocyte growth factor/c-Met signaling pathway accelerates early stages of N-nitrosodiethylamine induced hepatocarcinogenesis. *Cancer Res*. 2007;67(20):9844–9851.
24. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov*. 2008;7(6):504–516.
25. Osipo C, Miele L. Hedgehog signaling in hepatocellular carcinoma: novel therapeutic strategy targeting hedgehog signaling in HCC. *Cancer Biol Ther*. 2006;5(2):238–239.
26. Liang CM, Chen L, Hu H, Ma HY, Gao LL, Qin J. Chemokines and their receptors play important roles in the development of hepatocellular carcinoma. *World J Hepatol*. 2015;7(10):1390–1402.
27. Sutton A, Friand V, Brulé-Donneger S, et al. Stromal cell-derived factor-1/chemokine (C-X-C motif) ligand 12 stimulates human hepatoma cell growth, migration, and invasion. *Mol Cancer Res*. 2007;5(1):21–33.
28. Liu H, Pan Z, Li A, et al. Roles of chemokine receptor 4 (CXCR4) and chemokine ligand 12 (CXCL12) in metastasis of hepatocellular carcinoma cells. *Cell Mol Immunol*. 2008;5(5):373–378.
29. Fu J, Zhang Z, Zhou L, et al. Impairment of CD4⁺ cytotoxic T cells predicts poor survival and high recurrence rates in patients with hepatocellular carcinoma. *Hepatology*. 2013;58:139–149.
30. Cabrera R, Szabo G. Another armed CD4⁺ T cell ready to battle hepatocellular carcinoma. *Hepatology*. 2013;58(1):1–3.
31. Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res*. 2005;65(6):2457–2464.
32. Shen P, Wang A, He M, Wang Q, Zheng S. Increased circulating Lin (–/low) CD33 (+) HLA-DR (–) myeloid-derived suppressor cells in hepatocellular carcinoma patients. *Hepatol Res*. 2014;44(6):639–650.
33. Qin L-X. Inflammatory immune responses in tumor microenvironment and metastasis of hepatocellular carcinoma. *Cancer Microenviron*. 2012;5(3):203–209.
34. Chew V, Chen J, Lee D, et al. Chemokine-driven lymphocyte infiltration: an early intratumoural event determining long-term survival in resectable hepatocellular carcinoma. *Gut*. 2012;61(3):427–438.
35. Bray SM, Vujanovic L, Butterfield LH. Dendritic cell-based vaccines positively impact natural killer and regulatory T cells in hepatocellular carcinoma patients. *Clin Dev Immunol*. 2011;249281:1–11.
36. Kakumu S, Ito S, Ishikawa T, et al. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. *J Gastroenterol Hepatol*. 2000;15(4):431–436.
37. Tada F, Abe M, Hirooka M, et al. Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Int J Oncol*. 2012;41(5):1601–1609.
38. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. *Cancer Cell*. 2004;5:215–219.
39. Villanueva A, Newell P, Chiang D, Friedman S, Llovet JM. Genomics and signaling pathways involved in the pathogenesis of HCC. *Semin Liver Dis*. 2007;27(1):55–76.
40. Kancherla V, Abdullazade S, Matter MS, et al. Genomic analysis revealed new oncogenic signatures in TP53-mutant hepatocellular carcinoma. *Front Genet*. 2018;9(2):1–13.
41. Mao R, Liu J, Liu G, et al. Whole genome sequencing of matched tumor, adjacent non-tumor tissues and corresponding normal blood samples of hepatocellular carcinoma patients revealed dynamic changes of the mutations profiles during hepatocarcinogenesis. *Oncotarget*. 2017;8(16):26185–26199.
42. Andrade RC, de Lima MAF, de Faria PAS, Vargas FR. TP53 germline and somatic mutations in a patient with fibrolamellar hepatocellular carcinoma. *Fam Cancer*. 2018;17:119–122.
43. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet*. 2002;31:339–346.
44. Grisham JW. Molecular genetic alterations in primary hepatocellular neoplasms: hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma. Coleman W.B, Tsongalis G.J, editors. *In: The Molecular Basis of Human Cancer*. Totowa, NJ: Humana Press; 2002:269–346.
45. Buendia MA. Genetics of hepatocellular carcinoma. *Semin Cancer Biol*. 2000;10:185–200.
46. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer*. 2006;6:674–687.
47. Dhanasekaran R, Limaye A, Cabrera R. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis, and therapeutics. *Hepatic Med: Evid Res*. 2012;4:19–37.
48. Murakami Y, Saigo K, Takashima H, et al. Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. *Gut*. 2005;54(8):1162–1168.
49. Tarn C, Lee S, Hu Y, Ashendel C, Andrisani OM. Hepatitis B virus X protein differentially activates RASRAF-MAPK and JNK pathways in X-transforming versus non-transforming AML12 hepatocytes. *J Biol Chem*. 2001;276:34671–34680.
50. Feitelson MA, Sun B, Satioglu Tufan NL, Liu J, Pan J, Lian Z. Genetic mechanisms of hepatocarcinogenesis. *Oncogene*. 2002;21:2593–2604.
51. Macdonald A, Crowder K, Street A, McCormick C, Saksela K, Harris M. The hepatitis C virus non-structural NS5A protein inhibits activating protein-1 function by perturbing ras-ERK pathway signaling. *J Biol Chem*. 2003;278:17775–17784.

52. Majumder M, Ghosh AK, Steele R, Ray R, Ray RB. Hepatitis C virus NS5A physically associates with p53 and regulates p21/waf1 gene expression in a p53-dependent manner. *J Virol.* 2001;75(3):1401–1407.
53. Roberts LR, Gores GJ. Hepatocellular carcinoma: molecular pathways and new therapeutic targets. *Semin Liver Dis.* 2005;25:212–225.
54. Breuhahn K, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. *Oncogene.* 2006;25:3787–3800.
55. Branda M, Wands JR. Signal transduction cascades and hepatitis B and C related hepatocellular carcinoma. *Hepatology.* 2006;43(5):891–902.
56. de La Coste A, Romagnolo B, Billuart P, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci U S A.* 1998;95:8847–8851.
57. Ishizaki Y, Ikeda S, Fujimori M, et al. Immunohistochemical analysis and mutational analyses of beta-catenin, Axin family and APC genes in hepatocellular carcinomas. *Int J Oncol.* 2004;24:1077–1083.
58. Merle P, de la Monte S, Kim M, et al. Functional consequences of frizzled-7 receptor overexpression in human hepatocellular carcinoma. *Gastroenterology.* 2004;127:1110–1122.
59. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol.* 1998;14:59–88.
60. Tanaka Y, Kanai F, Tada M, et al. Absence of PIK3CA hotspot mutations in hepatocellular carcinoma in Japanese patients. *Oncogene.* 2006;25:2950–2952.
61. Lee JW, Soung YH, Kim SY, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene.* 2005;24:1477–1480.
62. Sabatini DM. mTOR and cancer: insights into a complex relationship. *Nat Rev Cancer.* 2006;6:729–734.
63. Xiao Z, Shen J, Zhang L, Li M, Hu W, Cho C. Therapeutic targeting of noncoding RNAs in hepatocellular carcinoma: recent progress and future prospects. *Oncol Lett.* 2018. <http://dx.doi.org/10.3892/ol.2018.7758>.
64. Mohamed A, Ali-Eldin Z, Elbedewy T, El-Serafy M, Ali-Eldin F, AbdelAziz H. MicroRNAs and clinical implications in hepatocellular carcinoma. *World J Hepatol.* 2017;9(23):1001. <http://dx.doi.org/10.4254/wjh.v9.i23.1001>.
65. Zhou J, Yu L, Gao X, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol.* 2011;29(36):4781–4788. <http://dx.doi.org/10.1200/jco.2011.38.2697.2>.
66. Shen J, Siegel A, Remotti H, Wang Q, Santella R. Identifying microRNA panels specifically associated with hepatocellular carcinoma and its different etiologies. *Hepatoma Res.* 2016;2(6):151. <http://dx.doi.org/10.20517/2394-5079.2015.66>.
67. Gong J, He X, Tian D. Emerging role of microRNA in hepatocellular carcinoma. *Oncol Lett.* 2014;9(3):1027–1033. <http://dx.doi.org/10.3892/ol.2014.2816>.
68. Lu M, Kong X, Wang H, Huang G, Ye C, He Z. A novel microRNAs expression signature for hepatocellular carcinoma diagnosis and prognosis. *Oncotarget.* 2017;8(5). <http://dx.doi.org/10.18632/oncotarget.14452>.
69. Chen G, Huang W, Wang H, et al. Lower expressed miR-198 and its potential targets in hepatocellular carcinoma: a clinic pathological and in silico study. *Onco Targets Ther.* 2016;9:5163–5180. <http://dx.doi.org/10.2147/ott.s108828>.
70. Sartorius K. The biological and diagnostic role of miRNA's in hepatocellular carcinoma. *Front Biosci.* 2018;23(9):1701–1720. <http://dx.doi.org/10.2741/4668>.
71. Lee MH, Yang HI, Lu SN, et al. Polymorphisms near the IFNL3 gene associated with HCV RNA spontaneous clearance and hepatocellular carcinoma risk. *Sci Rep.* 2015;5:17030.
72. Vivekanandan P, Singh OV. High-dimensional biology to comprehend hepatocellular carcinoma. *Expert Rev Proteomics.* 2008;5(1):45–60.
73. Chauhan R, Lahiri N. Tissue- and serum-associated biomarkers of hepatocellular carcinoma. *Biomark Cancer.* 2016;8(S1):37–55.
74. Lou J, Zhang L, Lv S, Zhang C, Jiang S. Biomarkers for hepatocellular carcinoma. *Biomark Cancer.* 2017;1–9.
75. Berretta M, Cavaliere C, Alessandrini L, et al. Serum and tissue markers in hepatocellular carcinoma and cholangiocarcinoma: clinical and prognostic implications. *Oncotarget.* 2017;8(8):14192–14220.
76. Clark P. Serum markers for hepatocellular carcinoma. *Clin Liver Dis.* 2016;8(2):29–33.
77. Ye Q-H, Qin L-X, Forgues M, et al. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervise machine learning. *Nat Med.* 2003;9:416–423.
78. Chauhan R, Lahiri N. Tissue- and serum-associated biomarkers of hepatocellular carcinoma. *Biomark Cancer.* 2016;8(suppl 1):37–55.
79. Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer.* 2007;43:979–992.
80. Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology.* 2004;127:108–112.
81. Camaggi CM, Zavatto E, Gramantieri L, et al. Serum albumin-bound proteomic signature for early detection and staging of hepatocarcinoma: sample variability and data classification. *Clin Chem Lab Med.* 2010;48(9):1319–1326.
82. Kragh-Hansen U, Chuang VT, Otagiri M. Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. *Biol Pharm Bull.* 2002;25:695–704.
83. Mathé C, Sagot MF, Schiex T, Rouzé P. Survey and summary: current methods of gene prediction, their strengths and weaknesses. *Nucleic Acids Res.* 2002;30(19):4103–4117.
84. Maji S, Garg D. Progress in gene prediction: principles and challenges. *Curr Bioinform.* 2013;8(2):1–18.
85. Iddamalgoda L, Das PS, Aponso A, Sundararajan VS, Suravajhala P, Valadi JK. Data mining and pattern recognition models for identifying inherited diseases: challenges and implications. *Front Genet.* 2016;10(7):136.
86. Lafaro KJ, Demirjian AN, Pawlik TM. Epidemiology of hepatocellular carcinoma. *Surg Oncol Clin N Am.* 2015;24(1):1–17.
87. Cho W. Cancer biomarker discovery: the contribution of “omics”. *BIOforum Eur.* 2007;11(9):35–37.
88. Lee J-S, Kim JH, Park Y-Y, Mills GB. Systems biology approaches to decoding the genome of liver cancer. *Cancer Res Treat.* 2011;43(4):205.
89. Malaguarnera G, Bertino G, Vacante M, Malaguarnera M. Hepatocellular carcinoma markers in the omics era: the glycomic analysis. *Hepatobiliary Surg Nutr.* 2014;3(6):407–409.
90. Wu Z, Galmiche A, Liu J, et al. Neurotensin regulation induces overexpression and activation of EGFR in HCC and restores response to erlotinib and sorafenib. *Cancer Lett.* 2017;388:73–84.
91. Zucman-Rossi J, Laurent-Puig P. Genetic diversity of hepatocellular carcinomas and its potential impact on targeted therapies. *Pharmacogenomics.* 2007;8(8):997–1003.
92. Paolillo C, Lordin E, Fortina P. Next generation sequencing in cancer: opportunities and challenges for precision cancer medicine. *Scand J Clin Lab Invest Suppl.* 2016;245:S84–S91.
93. Ma C, Zhang Q, Greten TF. Non-alcoholic fatty liver disease promotes hepatocellular carcinoma through direct and indirect effects on hepatocytes. *FEBS J.* 2017.
94. Lou J, Zhang L, Lv S, Zhang C, Jiang S. Biomarkers for hepatocellular carcinoma. *Biomark Cancer.* 2017.