GENOMIC, EPIGENOMIC AND PROTEOMIC LANDSCAPING OF HEPATOCELLULAR CARCINOMA

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common and fatal malignancy in humans and proves to be the third most common cause of cancer-related death. Thus, HCC contributes to major international health problem because its incidence is exponentially increasing in many countries. One of the main reasons for the lethality of HCC is the lack of diagnostic markers for early detection of the disease. At late stages, HCC shows a high clinical heterogeneity with poor prognosis i.e. high tumor recurrence is observed in 60-70% of cases within 5 years after surgery. One of the major reasons is that most patients with HCC were diagnosed at advanced stages. It is crucial to find out new therapeutic targets and novel diagnostic biomarkers for the early diagnosis and timely treatment of HCC and to develop preventive strategies and therapeutic interventions based on an improved understanding of molecular hepato carcinogenesis. Therefore, it is still urgent to further explore the exact molecular mechanisms of the development, progression, invasion, and metastasis of HCC. It has been shown that both genetic and epigenetic alterations are crucial for the initiation of HCC, thus making epigenetics a promising and attractive field for identifying the subset of patients at a high risk of recurrence and with dismal survival outcomes. However, the underlying molecular mechanisms remain unknown. Thus, it is urgent and important to dig the hub molecules and to uncover the key molecular mechanisms. Due to the advances made in research based on next generation sequencers, it is now possible to detect and analyse epigenetic abnormalities associated with cancer. In this review article we are trying to explore previously reported to play key role in HCC development and progression such as, DNA methylation, various histone modifications, chromatin remodelling, and non-coding RNA associated gene silencing are considered to be transcriptional regulatory mechanisms associated with gene expression changes.

Keywords: *Hepatocellular Carcinoma,DEGs ,miRNAs,lncRNA*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequently occurring and fatal hepatic malignancy as well as a major cause of cancer-related death worldwide. Due to its aggressive andheterogeneous nature, HCC pathogenesis is not fully understood till date. However, high rate of incidence and mortality is reported in Southeast Asia and Africa, where infection of hepatitis B virus is endemic [1]. Hepatitis B and Hepatitis C viruses are considered as causal agents for HCC.However, in these areas, majority of the cases are secondary to chronic liver cirrhosis. Some othermajor risk factors reported for HCC include aflatoxin exposure, tobacco use, non-alcoholic fatty liver disease, metabolic syndrome, and various carcinogens. Due to lethality of HCC the long-termsurvival rate of HCC patients remains low worldwide. The mean survival time of HCC patient is estimated approximately 6 – 20 months [2-4]. Although remarkable improvements are achieved inthe treatment of HCC such as liver transplantation, radical surgical resection, and interventional therapy, but treatment outcomes still remain unsatisfactory due to post-surgical recurrence and drug resistance [4,5]. At present, surgical resection is first-line treatment for HCC, even then, some of these patients experience

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recurrence. Systematic treatments that are personalized for each patient are regarded as therapy options, but unfortunately, not all treatments are successful and poor prognosis remains a major problem. One of the causes for such high lethality of HCC is thatit is difficult to detect at early stages, and it is characterized by a high degree of malignancy, and poor prognosis, and rapid progression, only 10–20% of patients with HCC are eligible for surgical treatment [6]. Therefore, it is still urgent to further explore molecular mechanisms of the development, progression, invasion, and metastasis of HCC, which can help to develop biomarkersfor early diagnosis of HCC. There is an urgent need to better understand the molecular pathogenesis of HCC and explore novel therapies. Recent research has been undertaken to better understand molecules and pathways related to tumorigenesis. Based on an improved understanding of molecular hepato-carcinogenesis, novel biomarkers and therapeutic targets could be developed[6-8].

PROGRESSION OF LIVER CIRRHOSIS TOWARDS HEPATOCELLULAR CARCINOMA

Almost all patients with hepatocellular carcinoma (HCC) also have liver cirrhosis. The severity ofcirrhosis hampers effective treatment for HCC despite recent progress in the efficacy of anticancerdrugs for advanced stages of HCC. Studies reveal that ~70% of patients with HCC have hepatitisB virus (HBV) and/or hepatitis C virus (HCV) infection [4,5]. The literature suggests that geneticand epigenetic factors, such as microRNAs, play a role in liver cirrhosis and its progression to HCC, and that HBV- and HCV-encoded proteins appear to be involved in hepato-carcinogenesis.Chronic hepatitis caused by HBV infection is one of the main causes of HCC. Numerous studies have confirmed that HBV can activate a variety of signals to promote viral replication and inflammation progression and to accelerate hepato-carcinogenesis [5].

GENETICS AND HCC

Driver-Gene Candidates in HCC

In the past decades, there have been many reports of genes involved in HCC initiation and progression. 30 candidate driver genes [telomerase reverse transcriptase (TERT), catenin _1 (CTNNB1), tumor protein p53 (TP53), AT-rich interaction domain 2 (ARID2), axin 1 (AXIN1), TSC complex subunit 2 (TSC2), retinoblastoma protein 1 (RB1), activin A receptor type 2A (ACVR2A), bromo domain containing 7 (BRD7), cyclin dependent kinase inhibitor (CDKN)1A, menin 1 (MEN1), polypeptide N-acetyl galactosaminyl transferase 11 (GALN11), fibroblast growth factor 19 (FGF19), cyclin (CCN)D1, AT-rich interaction domain 1A (ARID1A), CDKN2A, CDKN2B, ribosomal protein S6 kinase, 90 kDa, polypeptide 3 (RPS6KA3), nuclear factor, erythroid 2 like 2 (NFE2L2), nuclear receptor co-repressor 1 (NCOR1), alcohol dehydrogenase 1B, _ polypeptide (ADH1B), Snf2-related CREB binding protein (CREBBP) activator protein (SRCAP), Fc receptor like 1 (FCRL1), phosphatase and tensin homolog (PTEN),heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), cytochrome P450 family were reported by Totoki *et al.*

Fujimoto *et al.* [53] identified 15 significantly mutated genes, including TP53, ERBB-receptor feedback inhibitor 1, Zinc family member 3, CTNNB1, glucoside xylosyltransferase 1, otopetrin 1, albumin (ALB), ATM serine/threonine kinase (ATM), zinc finger protein 226, ubiquitin specificpeptidase (USP)25,WW-domain-containing E3 ubiquitin protein ligase 1, immunoglobulin superfamily member 10, ARID1A and bromo-domain adjacent to zinc finger domain 2B, after sequencing and analysing the whole genomes of HCC samples, including HBV- or HCV- associated HCC [52-55].

Genes involved in HCC metastasis

Recurrence and metastasis are reported as causes of lethality and higher mortality rate in HCC patients. The identification of genes with a broad range of function in HCC metastasis would allowfor the control and/or prevention of metastasis, thus improving patient survival rate in HCC. A study reported genes such as *RHOC, GRN, VIM, DLG7, HLA-DRA, CLDN10, EFNA1, PDGFRA,*and *NDRG1,* in HCC metastasis. Among these genes *RHOC, VIM, DLG7,* and *CLDN10* functionas cell invasion regulators, and *GRN, PDGFRA,* and *NDRG1* function as cell growth regulators [6].

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Differentially expressed genes in HCC

For better understanding of molecular mechanisms involved in HCC occurrence and progression,recent studies are focused in the direction of gene expression analysis. A study revealed overexpression of the general transcription factor IIB (GTF2B) may contribute to HCC pathogenesis. Cao *et al.* reported that the overexpression of DDB1 and CUL4-associated factor 13is associated with poor survival in HCC. Wu *et al.* observed that the expression level of OCIAD2in the tumor tissues was much lower than that in the corresponding adjacent normal tissues, and OCIAD2 suppressed tumor growth and invasion. Furthermore, a recent study reported a total of 118 differentially expressed genes between very early HCC and cirrhotic tissue samples. A PPI network was constructed and top eight hub genes, including CDKN3, CDK1, CCNB1, TOP2A, CCNA2, CCNB2, PRC1, and RRM2, were identified and reported. High expressions of CDK1, CCNB1, TOP2A, CCNA2, PRC1, RRM2, CDKN3, and CCNB2 were associated with poorer overall survival in HCC patients. Some literature states expression levels of *CDK1*, *CCNB1*, *CCNB2*, *MAD2L1*, and *TOP2A* were up-regulated in HCC. The expression levels of *ACACB*, *IGF1*, and *EHHADH* were down-regulated in HCC [51,52]. These genes may be potentially utilized as therapeutic targets and prognostic biomarkers for HCC. Also, this data improves our understanding of the underlying mechanisms of HCC, although further investigations are warranted. A study confirmed that higher expression levels of PLK1, PRCC, PRPF4 and PSMA7co-related with poor prognosis [53-56].

NON-CODING RNAS AND HCC

miRNAs in HCC

Till date, numbers of studies have already stated regulatory roles of miRNAs in various diseases including multiple cancers. However, to add on to this, some studies explored emerging role of microRNAs as diagnostic markers and therapeutic targets in HCC. In HCC, miRNAs are involvedin carcinogenesis, progression, metastasis, and has been reported to be a factor in whether the cancer is susceptible to treatment. It has been reported that miR-34a-5p may inhibit the proliferation of HCC cells via regulating MCM2 expression. Additionally, miRNAs are also foundin exosomes and other extracellular vesicles, which suggest that they play a potential role in exosome-mediated cross-talk in cancer. Murakami and colleagues reported that some miRNAs are overexpressed in HCC compared to adjacent non-tumorous tissues and are linked to differentiation of HCC [14-17].

Differentially Expressed miRNAs in HCC

Recently, a study was conducted to focus on Differential expression analysis of miRNAs in HCC,and it reported 3 miRNAs (*miR-26a*, *miR-122*, and *miR-130a*) were down-regulated and other threemiRNAs (*miR-21*, *miR-93*, and *miR-221*) were up-regulated in HCC. miRNAs (*miR-21*, *miR-221,miR-222*6, *and miR-224*7) are consistently up-regulated in the tumors of HCC patients and were reported to dys-regulate proliferation and/or apoptosis. M*iR-122*11 and *miR-199a*6 are consistentlydown-regulated in HCC tumors [14-17].

Etiology	Up-regulated miRNAs	Reference
HCV	miR-10a, miR100, miR-122, miR-155, miR-224, miR-452, miR-1269	13,14,15
HBV	miR-21, miR-33a, miR-25a, miR-143, miR-148a, miR-221, miR331-3p, miR-602	16-23
NASH	miR-10b, miR-16, miR21, miR-23a, miR-31, miR-33,miR-155, miR-221/222, miR-93	36-39
Alcohol	miR-10b, miR-21, miR-500a, miR-532	40

Table 1 up regulated miRNAs in HCC

Etiology	Down-regulated miRNAs	Reference
HCV	miR-122, miR-130a, miR-139, miR-145, miR-198, miR-199a/b, miR-214	13,14
HBV	miR-15b, miR-29c, miR-101, miR-122, miR-132, miR-145, miR-148a, miR-152, miR-199a-5p, miR-205, miR-429	24-35

| NASH | miR-34a, miR-99a, miR-122, miR-199a/b-3p, miR-200a/b | 36,37 |
| Alcohol | miR-424, miR-3607, miR-139, miR-130a, miR-24-1, miR-29c, miR-101 | 40 |

Table 2 down regulated miRNAs in HCC

lnc RNAs in HCC

A recent research article reveals that the lncRNA H19 is down-regulated in HCC tissues comparedwith surrounding non-tumor tissues. The same study also suggests that treatment using a DNA de-methylating agent, such as 5-azacytidine, re-induces H19 expression and suppresses chemoresistance and tumorigenesis. Thus, this could be regarded as alternative therapy option forthe cases with chemoresistance. Furthermore, *Panzitt* and colleagues, in 2007, identified and reported up-regulated long non-coding RNA (HULC) [57]. This study also reports that the elevation of HULC by hepatitis B virus protein promotes hepatic cell proliferation by down- regulating p18. Recent whole-genome analyses detected many somatic mutation and copy numbervariation in lncRNAs observed in HCC. These findings imply that dysregulation of non-coding RNAs such as lncRNAs plays important roles in hepato carcinogenesis.

circular RNAs in HCC

Advances in high-throughput sequencing have revealed that, in cancer including HCC, the circRNA expression level changes, which suggests its role in carcinogenesis. Being aware of the role of various regulatory components in number of diseases including cancer, a study elucidated the role of circular RNAs in HCC. The research has demonstrated that circ_0021093 is overexpressed in HCC specimens compared to noncancerous counterparts, according to high- throughput circRNA sequencing. Furthermore, these findings also reveal that circ_0021093promotes cell growth and invasion but inhibits cell apoptosis through the miR-766-3p/MTA3 axis. However, by contrast, it is also reported that knockdown of circ_0021093 suppressed HCC progression. Thus, the circ_0021093/miR-766-3p/MTA3 regulatory axis may be an effective therapeutic target for HCC [57].

circRNA	Altered expression	Target miRNA	Reference
hsa_cir_0001649	Down-regulated	Undetermined	41
hsa_cir_0004018	Down-regulated	miR-30e, miR-92a-1,miR-647, miR-660	42
hsa_cir_0005986	Down-regulated	miR-129	43
circZKSCAN1	Down-regulated	Undetermined	44
cSMARCA5	Down-regulated	miR-17, miR-181b	45
circMTO1	Down-regulated	miR-9	46
hsa_cir_0005075	Up-regulated	miR-23a/b, miR-93, miR-581	47
ciRS-7(CDR1as)	Up-regulated	miR-7	48
Circ_0067934	Up-regulated	miR-1324	49
circHIPK3	Up-regulated	miR-124	50
circRNA_100338	Up-regulated	miR-141-3p	51

Table 3 differentially expressed circular RNAs in HCC

KEY PATHWAYS FOR HEPATOCELLULAR CARCINOMA

All the differentially expressed genes reported in various studies were found to be strongly associated with several biological processes, such as negative regulation of growth and p53 signalling pathway. DEGs such as CCNB1, CDC20, and CDK2 as well as classified under the categories of the p53 signalling pathway and the cell cycle were associated with HCC. Meanwhile, the metabolic pathway, protein processing in the endoplasmic reticulum and the thyroid cancer pathway may play vital roles in the progressionof HCC [53,56]. A study reported metabolic pathway, protein processing in the endoplasmic reticulum and thyroid cancer pathway to be the essential and most important mechanisms in the development of HCC. A closer examination of the Cell Growth and Death pathway revealed that the gene targets of all six differentially expressed miRNAs modulate TP53 signalling. However, the gene targets of all three up-regulated miRNAs are involved in apoptosis, whereas the gene targets

of all three down-regulated miRNAs are involved in cell cycle. Gene targets of all three up-regulated miRNAs (*miR-21*, *miR-93*, and *miR-221*), gene targets of all three down-regulated miRNAs (*miR- 26a*, *miR-122*, and *miR-130*) primarily target Genetic Information Processing, in particular, DNA replication and repair, transcription, cell growth, and nucleotide metabolism. miRNAs (*miR-26a*, *miR-122*, and *miR-130a*) were down-regulated in HCC, and their up-regulatedgene targets are primarily associated with aberrant cell proliferation that involves DNA replication,transcription and nucleotide metabolism other three miRNAs (*miR-21*, *miR-93*, and *miR-221*) wereup-regulated in HCC, and their down-regulated gene targets are primarily involved in metabolismand immune system processes [53,56].

EPIGENETICS AND HCC

DNA methylation and HCC
The regulation of gene expression by DNA methylation involves control of tissue-specific gene expression as well as the epigenetic phenomenon of genomic imprinting. Due to advance studies in epigenetic modulations it has been reported that DNA methylation in promoters is known for silencing genes in HCC. As a result of this silencing effect a high frequency of suppression of p16, E-Cadherin, RASSF1A, RUNX3, Suppressor of cytokine signalling 1(SOCS1), and other tumor suppressor genes have been observed and reported. Furthermore, KDM1A is up-regulated in HCCtissues and more KDM1A positive cells are observed in the higher tumor stage. It is also reportedthat high ARID1A expression is associated with poor survival in patients with HCC and promotesmetastases of HCC. In a study, Zhao et al. observed that methylation-induced ASPP1 and ASPP2silence promoted tumor growth in HCC, which might serve as potential treatment targets. Genome-wide methylation profiling by Villanueva et al. identified many genes that are aberrantly methylated in HCC, such as *RSSFA1*, *IGF2*, *APC*, *RASSF5*, *SFRP5*, *NEFH*, *SEPT9*, *EFNB2* and *FGF6* [56,57].

Histone modifications and HCC
Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), which is a core molecule of PRC2, possess catalytic activity specific for the tri-methylation of H3K27.As reported by a study, anincrease in EZH2 expression has been observed for some solid tumors such as melanoma. It is already demonstrated that EZH2-knockdown and EZH2 inhibitor treatment impaired cell growth and xeno graft tumor formation in HCC samples. According to literature mining, high level of EZH2 expression in tumors was observed in >50% of HCC patients. Thus, EZH2 can be considered as a potent therapeutic target for HCC. In HCC surgical samples, the expression of SUV39H1 and SETDB1 were higher in tumor tissues than those in corresponding non-tumor tissues. High levels of SUV39H1 expression have been shown to correlate with cancer recurrence.Loss-of-function of SUV39H1, but not SETDB1, inhibits cell growth ability and tumorigenicity in HCC cells [57].

Chromatin Remodelling and HCC
Chromatin remodelling is a molecular mechanism that allows modification of the architecture to enable regulation at the gene expression level. There are four families of ATP-dependent chromatin-remodelling factors (SWI/SNF, ISWI, CHD, and INO80). Among these families, the SWI/SNF family is a nucleosome remodelling complex, consisting of a large complex of subunits that is involved in ATP-dependent removal of nucleosomes or suppression of transcription. The human SWI/SNF family has two distinct complexes, BRG1/hBRM-associated factors (BAF) and poly bromo-associated BAF (PBAF). Recent whole-genome sequencing analysis revealed that multiple chromatin regulators, including ARID1A, ARID1B, ARID2, MLL and MLL3, were frequently mutated, somatic mutations or indels were detected in at least one of these chromatin regulators in approximately 50% of HCC patients [57].

PROTEINS AND HCC
Role of TET proteins in HCC
Ten- eleven translocation (TET) family perform oxidation of 5mC to 5 hydroxy methyl cytosine(5hmC). Among the three TET genes, TET1 and TET2 expression levels have frequentlybeen observed to be low in hepatocellular carcinoma (HCC) tissues. The modulation of TET1 alsocorrelates with microRNAs in a post- transcriptional regulatory process. Some studies also reported decreased expression of TET proteins and lower 5hmC levels are

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generally observed in these tumors and these alterations are associated to unfavourable clinical outcome in HCC patients[8]. Of interest, it has been also demonstrated that HBV infection was associated with global genomic 5hmC decrease in HCC samples. Additionally, recent studies revealed that 5hmC levels are down- regulated in HCC tissues and cell lines. Combined with the reported results, identification of 5hmC signatures in HCC tissues and in circulating cell- free DNA will certainly contribute to early detection and should help to design therapeutic strategies against HCC. 5hmC might also be a novel prognostic biomarker of HCC [8].

Role of RBBP5 in HCC

Retinoblastoma Binding Protein, RBBP5 was significantly up regulated in HCC tissues and cells. High RBBP5 expression was found to be associated with up-raised level of AFP, advanced TNM stage, high Ki-67 expression, larger tumor size, and poor prognosis. In a study, it was concluded that knockdown of RBBP5 significantly inhibited proliferation of HCC cells through cell cycle arrest [12]. Previous studies have shown that RBBP5 is up-regulated in some types of human cancers including glioma and multiple myeloma. Overexpression of RBBP5 promotes cell cycle progression and proliferation and induces chemotherapy resistance of cancer cells. A study confirms that high RBBP5 expression was associated with aggressive behaviour of HCC. RBBP5 was an independent prognostic indicator of survival of HCC patients, which was in agreement with previous study that glioma patients with high RBBP5 expression had worse prognosis. Low RBBP5 expression inhibits cell cycle progression in the process of HCC cell proliferation. Furthermore, inhibition of RBBP5 expression was found to enhance the sensitivity of HCC cells to DOX. These results indicate that RBBP5 plays an important role in the progression of HCC and may be a potential therapeutic target for HCC [12].

CONCLUSION

Many epigenetic, genomic, transcriptomic and proteomic abnormalities have been reported in different studies worldwide concerning HCC at a high frequency and the importance of these observations cannot be doubted. Currently, epigenomic changes are being used as a biomarker for diagnosis and in clinical trials of epigenetic drugs. The clinical application to HCCs utilizing epigenetic therapeutic agents has only just begun, and future developments are expected to occur. As the future development of therapy targeting genetic and epigenetic abnormalities is an important and urgent issue, it is believed that the importance of epigenetic drug discovery research will only continue to increase. Although recent advancements in functional genomics have increased our knowledge of HCC tremendously, our understanding of the molecular mechanisms leading to the disease still remains largely fragmentary. However, we need more experiments to investigate these novel, key and hub genes which might help to develop novel potential biomarkers and therapies for HCC.

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