GENOMIC, EPGENOMIC 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 and heterogeneous 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 other major 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-term survival 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 in the 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
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 that it 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 biomarkers for 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 hepatocellular carcinoma, 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 of cirrhosis hampers effective treatment for HCC despite recent progress in the efficacy of anticancer drugs for advanced stages of HCC. Studies reveal that ~70% of patients with HCC have hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infection [4,5]. The literature suggests that genetic and 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 hepatocarcinogenesis. 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 hepatocarcinogenesis [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)1D, 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 (NCO1), 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 specific peptidase (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 allow for 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 function as cell invasion regulators, and GRN, PDGFRA, and NDRG1 function as cell growth regulators [6].

Volume I Issue I
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Page 130
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 OCIAD2 in the tumor tissues was much lower than that in the corresponding adjacent normal tissues, and OCIAD2 suppressed tumor growth and invasion. Additionally, this data improves our understanding of molecular mechanisms involved in HCC occurrence and progression, as well as contributing to the development of new therapeutic strategies.

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 involved in 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 found in 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 three miRNAs (miR-21, miR-93, and miR-221) were up-regulated in HCC. miRNAs (miR-21, miR-221, miR-2226, and miR-2247) are consistently up-regulated in the tumors of HCC patients and were reported to dys-regulate proliferation and/or apoptosis. MiR-12211 and miR-199a6 are consistently down-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, miR155, miR221/222, 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] |
January targets of all three upregulated miRNAs modulate TP53 signalling. However, HCC. A closer examination of the Cell Growth and Death pathway revealed 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 number variation in IncRNAs observed in HCC. These findings imply that dysregulation of non-coding RNAs such as IncRNAs plays important roles in hepatic 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_0021093 promotes 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].

**Table 2 Down regulated miRNAs in HCC**

| IncRNAs in HCC | miR-34a, miR-99a, miR-122, miR-199a/b-3p, miR-200a/b |
|----------------|------------------------------------------------------|
| Alcohol        | miR-424, miR-3607, miR-139, miR-130a, miR-24-1, miR-29c |
|                | miR-101                                               |

| Table 3 Differentially expressed circular RNAs in HCC |
|--------------------------------------------------------|
| **circRNA** | **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 |
| circMT01 | 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 |

**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 progression of 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-regulated gene 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) were up-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 advances in epigenetic modulations it has been reported that DNA methylation in promotors 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 HCC tissues and more KDM1A positive cells are observed in the higher tumor stage. It is also reported that high ARID1A expression is associated with poor survival in patients with HCC and promotes metastases 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 xenograft 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 methylcytosine(5hmC). Among the three TET genes, TET1 and TET2 expression levels have frequently been observed to be low in hepatocellular carcinoma (HCC) tissues. The modulation of TET1 also correlates with microRNAs in a post-transcriptional regulatory process. Some studies also reported decreased expression of TET proteins and lower 5hmC levels are
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.

REFERENCES
1) Yehia, M., Kamal, S. & Abdelaal, A. Evaluation of the diagnostic and therapeutic roles of non- coding RNA and cell proliferation related gene association in hepatocellular carcinoma. 706, 97–105 (2019).
2) Oncology, S. Prognostic Value of DEPDC1 Expression in Tumor and Non-tumor Tissue of Patients With Hepatocellular Carcinoma. 4430, 4423–4430 (2019).
3) Cao, H. et al. High FOXK1 expression correlates with poor outcomes in hepatocellular carcinoma and regulates stemness of hepatocellular carcinoma cells. Life Sci. 228, 128–134 (2019).
4) Cao, L. et al. A primary splenic angiosarcoma hepatic metastasis after splenectomy and its genomic alteration profi le. 28, 1–7 (2019).
5) Kanda, T., Goto, T., Hirotsu, Y., Moriyama, M. & Omata, M. Molecular mechanisms driving progression of liver cirrhosis towards hepatocellular carcinoma in chronic hepatitis B and C infections: A review. Int. J. Mol. Sci. 20, (2019).
6) Iizuka, N., Oka, M., Tsunedomi, T. & Oka, M. Translational microarray systems for outcome prediction of hepatocellular carcinoma. Cancer Sci. 99, 659–665 (2008).
7) Journal, A. I. et al. Expression tendency and prognostic value of TCF21 in hepatocellular carcinoma. Artif. Cells, Nanomedicine, Biotechnol. 47, 1466–1470 (2019).
8) Wang, P. Role of ten - eleven translocation proteins and 5 - hydroxymethylcytosine in
hepatocellular carcinoma. 1–11 (2019). doi:10.1111/cpr.12626
9) Midorikawa, Y., Makuuchi, M., Tang, W. & Aburatani, H. Microarray-based analysis for hepatocellular carcinoma: From gene expression profiling to new challenges. World J. Gastroenterol. 13, 1487–1492 (2007).
10) El Jabbour, T., Lagana, S. M. & Lee, H. Update on hepatocellular carcinoma: Pathologists’ review.
11) World J. Gastroenterol. 25, 1653–1665 (2019).
12) Kit, S. Association of CTLA-4 tagging polymorphisms and haplotypes with hepatocellular carcinoma risk. 0, 15–20 (2019).
13) Zhou, H. et al. Retinoblastoma Binding Protein 5 Correlates with the Progression in HepatocellularCarcinoma. 2018, 1–10 (2018).
14) Dragomir M, Calin GA. Circular RNAs in Cancer -Lessons Learned From microRNAs. Front Oncol. 2018 May 28;8:179.
15) Gong J, He XX, Tian A. Emerging role of microRNA in hepatocellular carcinoma (Review). OncolLett. 2015 Mar;9(3):1027-1033.
16) Bach DH, Hong JY, Park HJ, Lee SK. The role of exosomes and miRNAs in drug-resistance of cancer cells. Int J Cancer. 2017 Jul 15;141(2):220-230.
17) He S, Zhang DC, Wei C. MicroRNAs as biomarkers for hepatocellular carcinoma diagnosis and prognosis. Clin Res Hepatol Gastroenterol 2015;39:426-34.
18) Varnholt H, Drebber U, Schulze F, Wedemeyer I, Schirm-acher P, Dienes HP, Odenthal M. MicroRNA gene expression profile of hepatitis C virus-infected hepatocellular carcinoma. Hepatology2008; 47:1223-1232
19) Diaz G, Melis M, Tice A, Kleiner DE, Mishra L, Zamboni F, Farci P. Identification of microRNAs specifically expressed in hepatitis C virus-associated hepatocellular carcinoma. Int J Cancer 2013;133:816-824.
20) Zhang Y, Wei W, Cheng N, Wang K, Li B, Jiang X, Sun S. Hepatitis C virus-induced up-regulationof microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. Hepatology. 2012Nov;56(5):1631-40.
21) Coppola N, Potenza N, Pisaturro M, Mosca N, Tonziello G, Signoriello G, Messina V, Sagnelli C, Russo A, Sagnelli E. Liver microRNA hsa-miR-125a-5p in HBV chronic infection: correlation with HBV replication and disease progression. PLoS One2013; 8: e65336
22) Zhang X, Liu S, Hu T, Liu S, He Y, Sun S. Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. Hepatology. 2009 Aug;50(2):490-9.
23) Yuan K, Lian Z, Sun B, Clayton MM, Ng IO, Feitelson MA. Role of miR-148a in hepatitis B associated hepatocellular carcinoma. PLoS ONE 2012; 7: e35331.
24) Qiu X, Dong S, Qiao F, Lu S, Song Y, Lao Y, et al. HBx-mediated miR-21 upregulation repressestumor-suppressor function of PDCD4 in hepatocellular carcinoma. Oncogene. 2013; 32: 3296–305.
25) Chen JJ, Tang YS, Huang SF, Ai JG, Wang HX, Zhang LP. HBx protein-induced upregulation of microRNA-221 promotes aberrant proliferation in HBV-related hepatocellular carcinoma bytargeting estrogen receptor-α. Oncol Rep. 2015; 33: 792–8.
26) Selitsky SR, Dinh TA, Toth CL, Kurtz CL, Honda M, Struck BR, Kaneko S, Vickers KC, LemonSM, Sethupathy P. Transcriptomic Analysis of Chronic Hepatitis B and C and Liver Cancer Reveals MicroRNA-Mediated Control of Cholesterol Synthesis Programs. MBio. 2015 Dec;8(6):e01500-15.
27) Cao Y, Chen J, Wang D, Peng H, Tan X, Xiong D et al. Upregulated in Hepatitis B virus-associated hepatocellular carcinoma cells, miR-331-3p promotes proliferation of hepatocellular carcinoma cells by targeting ING5. Oncotarget 2015; 6: 38093–38106
28) Yang L, Ma Z, Wang D, Zhao W, Chen L, Wang G. MicroRNA-602 regulating tumor suppressor gene RASSF1A is overexpressed in hepatitis B virus-infected liver and hepatocellular carcinoma. Cancer Biol Ther 2010; 9: 803–808.
29) Wei X, Xiang T, Ren G, Tan C, Liu R, Xu X, Wu Z. miR-101 is down-regulated by the hepatitis B virus x protein and induces aberrant DNA methylation by targeting DNA methyltransferase 3A. Cell Signal. 2013 Feb;25(2):439-46.
30) Li C, Wang Y, Wang S, Wu B, Hao J, Fan H, Ju Y, Ding Y, Chen L, Chu X, Liu W, Ye X, Meng
31) S. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. J...
32] Wei X, Tan C, Tang C, Ren G, Xiang T, Qiu Z et al. Epigenetic repression of miR-132 expression by the hepatitis B virus x protein in hepatitis B virus-related hepatocellular carcinoma. Cell Signal 2013; 25: 1037–1043

33] Bandopadhyay M, Banerjee A, Sarkar N, Panigrahi R, Datta S, Pal A et al. Tumor suppressor micro RNA miR-145 and onco micro RNAs miR-21 and miR-222 expressions are differentially modulated by hepatitis B virus x protein in malignant hepatocytes. BMC Cancer 2014; 14: 721.

34] Xu X, Fan Z, Kang L, Han J, Jiang C, Zheng X, Zhu Z, Jiao H, Lin J, Jiang K, Ding L, Zhang H, Cheng L, Fu H, Song Y, Jiang Y, Liu J, Wang R, Du N, Ye Q. Hepatitis B virus x protein represses miRNA-148a to enhance tumorigenesis. J Clin Invest. 2013 Feb;123(2):630-45.

35] Wu CS, Yen CJ, Chou RH, Chen JN, Huang WC, Wu CY, Yu YL. Downregulation of microRNA-15b by hepatitis B virus X enhances hepatocellular carcinoma proliferation via fucosyltransferase2-induced Globo H expression. Int J Cancer. 2014 Apr 1;134(7):1638-47.

36] Huang J, Wang Y, Guo Y, Sun S. Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. Hepatology. 2010 Jul;52(1):60-70.

37] Zhang T, Zhang J, Cui M, Liu F, You X, Du Y, Gao Y, Zhang S, Lu Z, Ye L, Zhang X. Hepatitis B virus X protein inhibits tumor suppressor miR-205 through inducing hypermethylation of miR-205 promoter to enhance carcinogenesis. Neoplasia. 2013 Nov;15(11):1282-91.

38] Liu FY, Zhou SJ, Deng YL, Zhang ZY, Zhang EL, Wu ZB, Huang YZ, Chen XP. MiR-216b is involved in pathogenesis and progression of hepatocellular carcinoma through HBx-miR-216b-IGF2BP2 signaling pathway. Cell Death Dis. 2015 Mar 5;6:e1670.

39] Xiong Y, Fang JH, Yun JP, Yang J, Zhang Y, Jia WH, Zhuang SM. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. Hepatology. 2010 Mar;51(3):836-45.

40] Fu X, Tan D, Hou Z, Hu Z, Liu G. miR-338-3p is down-regulated by hepatitis B virus X and inhibits cell proliferation by targeting the 3'-UTR region of CyclinD1. Int J Mol Sci. 2012;13(7):8514-39.

41] You X, Liu F, Zhang T, Li Y, Ye L, Zhang X. Hepatitis B virus X protein upregulates oncogene Rab18 to result in the dysregulation of lipogenesis and proliferation of hepatoma cells. Carcinogenesis. 2013 Jul;34(7):1644-52.

42] Gori M, Ariciello M, Balsano C. MicroRNAs in nonalcoholic fatty liver disease: novel biomarkers and prognostic tools during the transition from steatosis to hepatocarcinoma. Biomed Res Int. 2014;2014:741465.

43] Lasda E, Parker R. Circular RNAs: diversity of form and function. RNA. 2014 Dec;20(12):1829-42.

44] Szabo L, Salzman J. Detecting circular RNAs: bioinformatic and experimental challenges. Nat Rev Genet. 2016 17(11):679-92.

45] Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013 Mar 21;495(7441):333-8.

46] Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013 Mar 21;495(7441):384-8.

47] Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circularRNAetardscelcycloprogressionviaformignternarycomplexeswith p21and CDK2. Nucleic Acids Res. 2016 Apr 7;44(6):2846-58.

48] Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evanthal N, Memczak S, Rajewsky N, Kadener S. circRNAbiogenesiscompeteswith pre-mRNAsplicing. Mol Cell. 2014Oct 2;56(1):55-66.

49] Qin M, Liu G, Huo X, Tao X, Sun X, Ge Z, Yang J, Fan J, Liu L, Qin W. Hsa_circ_0001649: A circular RNA and potential novel biomarker for hepatocellular carcinoma. Cancer Biomark. 2016;16(1):161-9.

50] Fu L, Yao T, Chen Q, Mo X, Hu Y, Guo J
Screening differential circular RNA expression profiles reveal that hsa_circ_0004018 is associated with hepatocellular carcinoma. Oncotarget. 2017 Apr 6;8(35):58405-58416.

51) Fu L, Chen Q, Yao T, Li T, Ying S, Hu Y, Guo J. Hsa_circ_0005986 inhibits carcinogenesis by acting as a miR-129-5p sponge and is used as a novel biomarker for hepatocellular carcinoma. Oncotarget. 2017 Jul 4;8(27):43878-43888.

52) Yao Z, Luo J, Hu K, Lin J, Huang H, Wang Q, Zhang P, Xiong Z, He C, Huang Z, Liu B, Yang Y. ZKSCAN1 gene and its related circular RNA (circZKSCAN1) both inhibit hepatocellular carcinoma cell growth, migration, and invasion but through different signaling pathways. Mol Oncol. 2017 Apr;11(4):422-437.

53) Yu J, Xu QG, Wang ZG, Yang Y, Zhang L, Ma JZ, Sun SH, Yang F, Zhou WP. Circular RNA cSMARCA5 inhibits growth and metastasis in hepatocellular carcinoma. J Hepatol. 2018 Jan 31. pii: S0168-8278(18)30055-2.

55) Wu, M., Liu, Z., Li, X., Zhang, A., Lin, D., & Li, N. (2019). Analysis of potential key genes in very early hepatocellular carcinoma. World Journal of Surgical Oncology, 17(1), 1–8. https://doi.org/10.1186/s12957-019-1616-6

56) Chen, Q. F., Xia, J. G., Li, W., Shen, L. J., Huang, T., & Wu, P. (2018). Examining the key genes and pathways in hepatocellular carcinoma development from hepatitis B virus-positive cirrhosis. Molecular Medicine Reports, 18(6), 4940-4950. https://doi.org/10.3892/mmr.2018.9494

57) Thurnherr, T., Mah, W. C., Lei, Z., Jin, Y., Rozen, S. G., & Lee, C. G. (2016). Differentially Expressed miRNAs in Hepatocellular Carcinoma Target Genes in the Genetic Information Processing and Metabolism Pathways. Scientific Reports, 6(August 2015), 1-12. https://doi.org/10.1038/srep20065

58) Gao, X., Wang, X., & Zhang, S. (2018). Bioinformatics identification of crucial genes and pathways associated with hepatocellular carcinoma. Bioscience Reports, 38(6), 1–8. https://doi.org/10.1042/BSR20181441

59) Tu, J., Chen, J., He, M., Tong, H., Liu, H., Zhou, B., ... Wang, Z. (2019). Bioinformatics analysis of molecular genetic targets and key pathways for hepatocellular carcinoma. OncoTargets and Therapy, 12, 5153–5162. https://doi.org/10.2147/OTT.S198802

60) Nakamura, M., Chiba, T., Kanayama, K., Kanzaki, H., Saifo, T., Kusakabe, Y., & Kato, N. (2019). Epigenetic dysregulation in hepatocellular carcinoma: an up-to-date review. Hepatology Research, 49(1), 3–13. https://doi.org/10.1111/hepr.13250