**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript No: 17789**

**Manuscript Type: TOPIC HIGHLIGHTS**

**2015 Advances in Hepatocellular Carcinoma**

**Genetic and epigenetic aspects of initiation and progression of hepatocellular carcinoma**

Kanda M *et al.* Novel genetic and epigenetic alteration of HCC

Mitsuro Kanda, Hiroyuki Sugimoto, Yasuhiro Kodera

**Mitsuro Kanda, Hiroyuki Sugimoto, Yasuhiro Kodera,** Department of Gastroenterological Surgery (Surgery II), Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

**Author contributions:** Kanda M wrote the manuscript; Sugimoto H and Kodera Y revised the manuscript for important intellectual content.

**Conflict of interest statement**: We have nothing to declare.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** **Mitsuro Kanda, MD, PhD,** Department of Gastroenterological Surgery (Surgery II), Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. m-kanda@med.nagoya-u.ac.jp

**Telephone:** +81-52-7442249

**Fax:** +81-52-7442255

**Received:** March 24, 2015

**Peer-review started:** March 27, 2015

**First decision:** April 24, 2015

**Revised:** May 8, 2015

**Accepted:** September 2, 2015

**Article in press:**

**Published online:**

**Abstract**

Hepatocellular carcinoma (HCC) is a primary cancer of the liver that is predominant in developing countries and is responsible for nearly 600000 deaths each year worldwide. Similar to many other tumors, the development of HCC must be understood as a multistep process involving the accumulation of genetic and epigenetic alterations in regulatory genes, leading to the activation of oncogenes and the inactivation or loss of tumor suppressor genes. Extensive research over the past decade has identified a number of molecular biomarkers, including aberrant expression of HCC-related genes and microRNAs. The challenge facing HCC research and clinical care at this time is to address the heterogeneity and complexity of these genetic and epigenetic alterations and to use this information to direct rational diagnosis and treatment strategies. The multikinase inhibitor sorafenib was the first molecularly targeted drug for HCC to show some extent of survival benefits in patients with advanced tumors. Although the results obtained using sorafenib support the importance of molecular therapies in the treatment of HCC, there is still room for improvement. In addition, no molecular markers for drug sensitivity, recurrence and prognosis are currently clinically available. In this review, we provide an overview of recently published articles addressing HCC-related genes and microRNAs to update what is currently known regarding genetic and epigenetic aspects of the pathogenesis of HCC and propose novel promising candidates for use as diagnostic and therapeutic targets in HCC.

**Key words:** Hepatocellular carcinoma; Oncogene; Tumor suppressor gene; MicroRNA; DNA methylation

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Despite the large number of studies dedicated to the molecular diagnosis of hepatocellular carcinoma (HCC), highly sensitive biomarkers of the initiation and progression of HCC still need to be identified. At the same time, the development of novel molecular targeting agents that can surpass the effect of the multikinase inhibitor sorafenib is much-anticipated. This review aimed to update our knowledge of genetic and epigenetic aspects of HCC by providing an overview of novel HCC-related genes and microRNAs as candidates for use as diagnostic and therapeutic targets in HCC.

Kanda M, Sugimoto H, Kodera Y. Genetic and epigenetic aspects of the initiation and progression of hepatocellular carcinoma. *World J Gastroenterol* 2015; In press

**INTRODUCTION**

Hepatocellular carcinoma (HCC) ranks among the most common cancers worldwide and is the third leading cause of cancer death, accounting for approximately 600000 deaths annually[1, 2]. Although the prevalence of HCC remains highest in eastern Asia and Africa, the incidence is steadily increasing in Western countries[3]. Controlling HCC is difficult, as recurrence or metastasis is quite common in patients, even after the application of successful topical therapies or curative hepatectomy[4,5]. In addition to the high prevalence of hepatitis C virus (HCV) infection as the main reason for the increasing incidence of HCC in Western countries, multiple etiologic factors, including chronic viral hepatitis B, alcohol consumption and aflatoxin, have been reported to lead to liver damage and to an increased incidence of HCC[6-8]. This situation leads not only to the requirement for a more complex clinical approach for HCC but also to high molecular variability of the disease[6].

Complex genetic and epigenetic alterations, chromosomal aberrations, mutations, and altered molecular pathways lead to the development of HCC[9,10]. Analyses of genetic and epigenetic alterations and different molecular pathways involved in the development of HCC help to identify potential new diagnostic tools and druggable targets[10-12]. The establishment of a robust molecular classification will pave the way for a more personalized treatment scheme for HCC[10,13]. In addition, targeted therapies for HCC are currently under intensive investigation, and accumulating evidence suggests that combination therapy targeting different pathways will potentiate anti-tumoral effects and will become the therapeutic approach adopted in the future[14,15]. The success of sorafenib has proven the concept that targeted therapy can confer survival benefits on patients with HCC, although these benefits have been limited thus far[10,16]. Although the heterogeneity of HCC makes it difficult to clarify the mechanism of cancer development and to develop effective therapeutics, molecules that are potentially responsible for the initiation or progression of HCC have been reported in succession[6,7,17]. In this article, we review current, updated knowledge regarding the molecular pathogenesis of HCC relevant to the development of novel diagnostic tools and therapeutic targets by providing an overview of known genetic and epigenetic alterations without confinement to specific gene groups, functions and pathways. Additionally, some representative genes were selected from each category by the following criteria: (1) highly-innovative genes or microRNA (miRNA); (2) data from relatively large number of patients; and (3) solid data by functional analyses or *in vivo* study, and important findings were summarized.

**FACILITATORS OF HCC TUMORIGENESIS; PUTATIVE ONCOGENES**

Abrupt changes in hepatocytes due to viral infection or exposure to hepatotoxic stress cause activation of oncogenes *via* point mutations, gene amplification, or changes in the promoter region, resulting in tumor development[18,19]. Subsequently, increased expression of several oncogenes can influence the survival of cancerous cells by suppressing apoptosis and regulating the cell cycle[18,20]. Thus, artificial inhibition of oncogenes or their upstream genes could be a novel therapeutic approach. Recently reported genes that are upregulated in HCC are summarized in Table 1[12,21-51]. Here, we cull some studies from the list and introduce cogent findings.

***Interleukin-6***

A relationship between chronic hepatic inflammation and HCC pathogenesis has been identified in previous epidemiologic studies[52]. Following infection with hepatitis viruses, Kupffer cells activate nuclear factor-kB and secrete inflammatory cytokines, including Interleukin (IL)*-6*[53]. Chang *et al*[43] investigated the roles of IL-6 in HCC and found that high serum levels of *IL-6* were significantly correlated with high expression levels of *OCT4*/*NANOG*, which is a pluripotent transcription factor. IL-6 stimulated the expression of an autocrine insulin-like growth factor-I and its receptor, depending on signal transducer and activator of transcription 3, which in turn stimulated stemness-related properties in both cell lines and xenografted mouse tumors. Inhibition of the insulin-like growth factor-I receptor *via* RNA interference or the use of an inhibitory agent significantly suppressed the *IL6*-induced stemness-related properties, both *in vitro* and *in vivo*[43]. Hence, it was indicated that *IL-6* plays an important role in the pathogenesis of HCC, which is initiated by chronic inflammation.

***Metastasis associated with colon cancer 1***

The metastasis associated with colon cancer 1 (*MACC1*)gene was first identified as the critical pro-metastatic factor in human colon cancer and has been demonstrated to activate the hepatocyte growth factor (*HGF*)/cellular MET proto-oncogene (*c-MET*) pathway through binding to the promoter of *c-MET*[54,55]. Yao *et al*[47] reported that *MACC1* expression was upregulated in HCC tissues and correlated with poor patient outcome. In functional analyses, *MACC1* repressed HCC cell apoptosis and promoted cell growth, effects which were abolished by the knockdown of *c-MET*. *MACC1* activated phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT signaling by sensitizing *HGF*/*c-MET* signaling and enhanced the *HGF*-driven phosphorylation of *BCL2*-associated agonist of cell death, caspase-9 and forkhead box O3 and inhibited their pro-apoptotic functions in HCC cells[47]. These authors also demonstrated that *MACC1* inhibited cell apoptosis and promoted HCC growth in an *in vivo* study[47]. Thus, *MACC1* may be a key factor in resistance to apoptosis in HCC.

***Sirtuin 1***

Sirtuin 1(SIRT1)is a member of the mammalian sirtuin family, which plays a critical role in the regulation of critical biological processes such as metabolism, aging, oncogenesis, and cancer progression[56]. *SIRT1* is the most well-characterized member of the sirtuin family and plays a key role in both cell death and survival, together with other p53 family members, forkhead box, sub-group O, transcription factors, and the nuclear factor-κB family[57]. Hao *et al*[28] found that SIRT1 was overexpressed in HCC cells and tissues and significantly promoted the migration and invasion ability of HCC cells by inducing the epithelial-mesenchymal transition. An *in vivo* study supported the oncogenic functions of *SIRT1* in enhancing metastasis[28]. Bae *et al*[58] found that knockdown of *SIRT1* inhibited cell growth by transcriptional deregulation of cell cycle proteins, leading to hypophosphorylation of *pRb*, which inactivated *E2F*/*DP1* target gene transcription, and thereby caused the G1/S cell cycle arrest. In addition, miR-29c was identified as a suppressor of *SIRT1* by comprehensive miRNA profiling and ectopic miR-29c expression recapitulated *SIRT1* knockdown effects in HCC cells[58]. On the contrary, Zhang *et al*[59] reported that *SIRT1* has anti-carcinogenic effects in HCC *via* the *AMPK*- mammalian target of rapamycin (mTOR) pathway. They evaluated the relationship between p53 mutations and activation of *SIRT1* in 252 patients with hepatitis B virus-positive HCC and found that activated *SIRT1* was associated with a longer recurrence free survival in HCC tissues harbouring mutant *p53*. Inhibition of *SIRT1* increased cell growth, bearing mutated *p53*, by suppressing AMPKactivity and enhancing *mTOR* activity[59]. The conflicting results from different papers indicated that *SIRT1* is multi-functional gene and its biological features are left unsolved.

***Targeting protein for Xenopus kinesin-like protein 2***

Targeting protein for Xenopus kinesin-like protein 2 (TPX2)is a microtubule-associated protein that affects spindle assembly in human cells and is upregulated in multiple tumor types[60,61]. Liu *et al*[35] revealed that TPX2 expression was elevated in HCC cells and tissues. Clinical analysis indicated that TPX2 expression in HCC tissues was evidently correlated with tumor stage, numbers and differentiation and with patient survival.TPX2expression was positively correlated with matrix metalloproteinase (MMP) 2 and *MMP9*, and knockdown of *TPX2* using small interfering RNA prominently reduced cell invasion and migration and decreased the expression of phosphorylated *AKT*, *MMP2* and *MMP9*. TPX2 was able to activate the kinase activity of Aurora A[35]. Furthermore, TPX2 upregulated the activation of the PI3K/AKT signaling pathway and may mechanistically be associated with the activity of MMP2, ultimately causing proliferation of cancer cells[35]. Huang *et al*[62] also investigated the expression and functions of TPX2 in HCC. They found that *TPX2* was overexpressed in HCC cells and tissues from 86 patients, and positive *TPX2* expression was significantly associated with vascular invasion, advanced stage and shortened overall and recurrence-free survival. Knockdown of TPX2 reduced cell proliferation and viability in HCC cells and slowed down tumor growth in a mouse xenograft model[62]. Taken together, these studies indicated that TPX2 may serve as a prognostic marker and promotes tumorigenesis and metastasis of HCC.

***Triggering receptors expressed on myeloid cells 1***

Triggering receptors expressed on myeloid cells 1 (TREM1) is an approximately 30-kDa transmembrane glycoprotein belonging to the immunoglobulin superfamily[63].TREM-1 consists of a single extracellular Ig-like region, a transmembrane domain, and a cytoplasmic domain with no signaling motifs. Activation of TREM-1 increases the secretion of tumor necrosis factor alpha (TNF-α), granulocyte-macrophage colony-stimulating-factor and certain chemokines and cytokines, suggesting that *TRM1* modulates inflammation[64]. Duan *et al*[23] evaluated *TREM1* expression in 322 patients with HCC and found that increased *TREM-1* expression was associated with recurrence and poorer survival and was identified as an independent prognostic factor for recurrence. In functional analyses, *TREM-1* significantly promoted the proliferation and invasion of HCC cells and inhibited their apoptosis[23]. The levels of the proinflammatory cytokines *IL-1b* and *TNF-α* were shown to be regulated by *TREM1* expression, suggesting that *TRM1* modulates inflammation in liver tissues[23]. Thus, *TREM1* may be a modulator of inflammation and serve as an important prognostic marker for HCC.

***Melanoma-associated antigens D2 and D4***

Melanoma-associated antigens (MAGE)are tumor‑specific antigens and have increasingly been utilized as therapeutic targets for immunotherapy[65]. MAGEproteins are classified into types I and II. Type I *MAGE* genes are located on the X‑chromosome and include MAGE‑A, B and C, which are expressed during germ cell development, but not by mature somatic cells[66]. By contrast, the localization, expression and oncological functions of type II MAGE proteins, which include MAGE‑D, E, F, G and H, are less clear[67,68]. The function of MAGE‑D2 is unclear; however, increased MAGE‑D2 expression may promote cancer cell adhesion to the vascular epithelium. Moreover, MAGE‑D2 has been reported to protect melanoma cells from tumor necrosis factor‑related apoptosis‑inducing ligand‑induced apoptosis[51]. MAGE-D4 is specifically expressed in normal brains and ovaries and reportedly contributes to the proliferation, migration, and invasion of tumor cells in breast cancer and oral squamous cell carcinoma[50]. We recently evaluated the clinical significance of the expression of MAGE‑D2 and D4 in HCC. Increased expression of both *MAGE-D2* and *D4* was associated with shorter postoperative survival of patients with HCC and was identified as an independent prognostic factor[50,51]. The expression levels of MAGE-D2 and D4in HCC tissues represent promising prognostic markers, and functional analyses are warranted.

**GENES SILENCED IN HCC, PUTATIVE TUMOR SUPPRESSORS**

Tumor suppressor genes (TSGs) are typically inactivated *via* mutation, deletion, or promoter methylation, which silences gene expression[69]. Inactivation of TSGs is a crucial process for HCC tumorigenesis and for the activation of oncogenes[70]. Table 2 provides an updated list of the genes known to be suppressed in HCC without hypermethylation[71-80], and overviews of some individual representative genes are provided.

***Aryl-hydrocarbon receptor nuclear translocator 2***

**Aryl-hydrocarbon receptor nuclear translocator (ARNT)-2**is a transcriptional regulator and a member of the basic helix-loop-helix/Per-ARNT-SIM superfamily, which are heterodimeric transcription factors that sense and respond to environmental signals or to physiological signals through their two PAS domains[81,82]. Li *et al*[79] showed that a high expression level of ARNT2 in HCC tissues was associated with prolonged overall and recurrence-free survival. Knockdown of *ARNT2* significantly increased the cell proliferation, invasion and migration ability, whereas forced expression of *ARNT2* inhibited the activities of HCC cells[79]. The influences of *ARNT2* on tumor growth were demonstrated in an *in vivo* study[79]. Hence, ARNT2 may be a potential biomarker and therapeutic target for HCC.

***B‑cell translocation gene 1***

B‑cell translocation gene 1 (*BTG1*) is a translocation partner of the c‑Myc gene in the case of B‑cell chronic lymphocytic leukemia and belongs to a family of antiproliferative genes[83,84]. *BTG1* is constitutively expressed in quiescent cells, and its expression is downregulated as cells enter the growth cycle[85]. In breast and ovarian cancer, artificial expression of BTG1 mediatedBcl‑2‑regulated apoptosis and suppressed the proliferation of cancer cells[85]. We recently reported that BTG1 expression was frequently reduced in HCC cells and tissues without point mutations or promoter hypermethylation[80]. Downregulation of BTG1 was significantly associated with poorer survival after curative hepatectomy[80]. Thus, BTG1 can be nominated as a novel predictor of the recurrence of HCC, and elucidation of the associated regulatory mechanisms is expected.

***Glutathione peroxidase 3***

Glutathione peroxidase 3 (GPx3) was found to be upregulated in acute-phase injury as an anti-oxidant to protect the organ from oxidative stress by detoxifying hydrogen peroxide and other free radicals[86]. Qi *et al*[75] demonstrated tumor suppressive functions of GPx3in HCC. Downregulation of GPx3 in HCC tissues was significantly correlated with advanced stages, vascular invasion and poor overall survival. In addition, the plasma GPx3 level was significantly associated with tumor size, the number of nodules and the recurrence rate. Artificial expression of GPx3 inhibited the proliferation and invasion of HCC cells both *in vitro* and *in vivo*. The tumor suppressive activity of GPx3 was mediated through the Erk-NFκB-SIP1 pathway[75]. Notably, GPx3 could be delivered into tumors by human-induced pluripotent stem cell-derived mesenchymal stem cells and exhibited tumor suppressive activity *in vivo*[75]. Hence, GPx3 is a candidate HCC-related TSG.

***Fibulin-5***

Fibulin-5 (FBLN5) is a member of the fibulin family, which is characterized by calcium-binding epidermal growth factor-like repeats and a globular carboxyl-terminal fibulin-type structure[87]. FBLN5 contains an integrin-binding arginyl-glycyl-aspartic acid motif, which binds to integrins and mediates endothelial cell adhesion[88]. FBLN5 regulates the extracellular matrix structure and has functions in fibrogenesis, angiogenesis and tumorigenesis[89]. Tu *et al*[76] revealed that reduced expression of *FBLN5* was frequently found in HCC cell lines and HCC tissues and was associated with multiple tumors, vascular invasion, advanced stages, and consequently, poorer outcomes. In addition, forced expression of FBLN5 significantly inhibited the migration and invasion of HCC cells. Knockdown of *MMP7* inhibited the migration and invasion of HCC cells, and restoring MMP7expression abrogated the tumor-suppressive effect of FBLN5[76]. It was indicated that FBLN5 acts as a TSG through the suppression of MMP7 in HCC.

**GENES THAT ARE EPIGENETICALLY SUPPRESSED IN HCC**

The results of epigenetic analyses and methylation and miRNA profiling are contributing to the knowledge derived from gene expression data and should not be forgotten in the molecular diagnosis of HCC[6,90]. Unique epigenetic changes have been identified in different genes in different tumor types, revealing site-specific methylation profiles[91-93]. In hepatocarcinogenesis, aberrant methylation of tumor-related genes does not only occur in advanced tumor stages, it is also recognized as a frequent and early event[1]. Promoter methylation of different types of TSGs has been demonstrated under premalignant conditions, such as chronic hepatitis or liver cirrhosis[94,95]. Moreover, the frequency of aberrant promoter methylation increases during the progression from precancerous lesions to HCC[1]. Therefore, epigenetic changes in preneoplastic or early neoplastic stages may serve as an indicator or biomarker for the screening of patients with an increased risk for HCC. Novel genes nominated as candidate methylated TSGs in HCC are listed in Table 3[11,96-106].

***Transcriptional intermediary factor 1 gamma***

Transcriptional intermediary factor 1 gamma (TIF1γ), alternatively referred to as tripartite motif 33 (TRIM33), is a member of the tripartite motif/RING finger, B-box proteins and is also a member of the E3 ubiquitin-ligase family[107]. *TIF1γ*plays a role in embryonic development, cell differentiation, transcriptional elongation, cell mitosis and the regulation of transforming growth factor beta (TGF-β) superfamily signaling[108]. Ding *et al*[97] investigated the expression and functions of *TIF1γ* in HCC. The expression level of TIF1γ in HCC was decreased in parallel with tumor stages and was significantly associated with patient outcome. TIF1γ inhibited the invasion and metastasis of HCC cells in both early- and advanced-stage HCC through the suppression of TGF-β/Smad signaling and Smad2/3/4 complex formation in HCC cells[97]. In addition, hypermethylation of CpG islands in theTIF1c promoter was found to be responsible for the downregulation of TIF1γ[97]. Thus, TIF1γ can be considered as a novel methylated TSG in HCC.

***Dihydropyrimidinase-like 3***

Dihydropyrimidinase-like 3 (DPYSL3)is a cell adhesion molecule and is actively expressed in normal tissues, including cardiac myocytes, the brain, pineal body, retina and smooth muscle, and is moderately expressed in various other tissues, including liver tissues[109,110]. DPYSL3 has been reported to be involved in the metastatic process in tumor cells in prostate and pancreatic cancer[111,112]. We found that DPYSL3 was downregulated in most HCC cell lines with DPYSL3 promoter hypermethylation, and its expression was restored after demethylation. DPYSL3 expression levels were inversely correlated with those of vascular endothelial growth factor (VEGF) and focal adhesion kinase (FAK) in both HCC cells and tissues[105]. Knockdown of *DPYSL3* enhanced the migration and invasion of HCC cells. Patients with extra-hepatic recurrences exhibited a significantly lower expression level of *DPYSL3* mRNA in HCC compared with those without extra-hepatic recurrences [105]. *DPYSL3* was shown to be a putative HCC tumor suppressor regulated by promoter hypermethylation.

***Dermatopontin***

Dermatopontin (DPT) is a tyrosine-rich acidic extracellular matrix (ECM) protein that binds to α3β1 integrin and to a proteoglycan receptor during cell adhesion[113]. *DPT* has multiple biological functions in physiological and pathological processes. It accelerates collagen fibrillogenesis and modulates the interaction between decorin and TGF-β[114]. Fu *et al*[98] found that downregulation of DPT was frequently observed in HCC tissues and was significantly associated with metastasis and a poor prognosis. Overexpression of *DPT* inhibited migration *in vitro* and intra-hepatic metastasis *in vivo*. They also found that *DPT* was silenced mainly due to promoter hypermethylation. Inhibition of *DPT* resulted in dysregulation of focal adhesion assembly, decreases of ras homolog family member A, FAK and c-Src tyrosine kinase phosphorylation *via* integrin signaling[98]. DPT is a putative methylated TSG mediating the metastatic ability of HCC.

***Prenyl diphosphate synthase subunit 2***

Prenyl diphosphate synthase subunit 2 (PDSS2) was identified in 2005[115]. It encodes the second subunit of prenyl diphosphate synthase, which is an essential enzyme involved in the biosynthesis of coenzyme Q10 (CoQ10), and PDSS2 determines the side-chain length of mammalian ubiquinones[116]. CoQ10 is synthesized from mevalonic acid in the liver and plays a vital role in the mitochondrial respiratory chain, pyrimidine nucleoside biosynthesis and the modulation of cell apoptosis[93]. Aberrant expression of PDSS2 in the liver may cause DNA damage and disrupt the cell cycle through inhibition of CoQ10 synthesis, leading to the initiation and progression of HCC[101]. Recently, we reported that *PDSS2* mRNA expression was frequently decreased in HCC cell lines and tissues[101]. The expression levels of PDSS2 were significantly correlated with those of hepatocyte nuclear factor 4α. *PDSS2* transcription in HCC cells with decreased PDSS2 expression accompanying hypermethylation was reactivated after treating these cells with a methylation inhibitor. *PDSS2* expression levels, relative to those in the uninvolved liver, decreased gradually from chronic hepatitis to cirrhosis[101]. Suppression of *PDSS2* was associated with a worse postoperative outcome. Decreased levels or hypermethylation of *PDSS2* may represent a novel biomarker of HCC.

**DYSREGULATED MICRORNAS IN HCC**

Growing evidence indicates a direct and interdependent link between epigenetic alterations and changes in miRNA expression, illustrating the complexity of epigenetic abnormalities in HCC[10,117]. MiRNAs are small RNA molecules, approximately 22 nucleotides long, that negatively control the expression of their target genes posttranscriptionally[118,119]. The importance of their role in cancer is increasingly being demonstrated because they can act as oncogenes or tumor suppressor genes[120]. In HCC, several miRNAs have been shown to be dysregulated. Some of these miRNAs function as oncogenes and inhibit apoptosis (miR-221), promote cell invasion (miR-9) or silence c-Met and, thus, inhibit migration and proliferation (miR23b)[121]. Others appear to show a TSG-like function (miR-101, 195, 122, 338) and are silenced in HCC[10]. MiRNA profiling is expected to be a source of additional information for better understanding the complex molecular heterogeneity of HCC, and consequently, providing a rationale for new therapeutic targets in HCC[117,118]. Here, we focus on some new discoveries regarding miRNAs involved in HCC progression (Table 4)[122-132].

***miR-128-2***

Zhuang *et al*[132] attempted to identify serum biomarkers for HCC patients using a TaqMan® Low-Density Array. As a result, miR-128-2 was found to be significantly upregulated in patients with portal vein tumor thrombosis. Patients with low levels of serum miR-128-2 were more likely to show a favorable prognosis[132]. In addition, the expression of miR-128-2 in HCC tissues was upregulated in HCC tissues compared with the corresponding non-cancerous liver tissues[132]. MiR-128-2 represents a promising serum screening tool for HCC.

***miR-148a***

Li *et al*[131] conducted microarray-based miRNA expression profiling in 100 HCC tissues. In patients categorized as presenting the subtype characterized by a cancer stem cell-like signature, the expression level of miR-148a was found to be significantly lower than in other patients. MiR-148a directly suppressed activin A receptor type 1 (ACVR1), a key receptor in the bone morphogenetic protein (BMP) signaling pathway, which regulates stem cell markers[133]. Administration of miR-148a reduced the aggressiveness of HCC cells both *in vitro* and *in vivo*[131]. MiR-148a may contribute to tumor progression *via* the miR-148a-*ACVR1*-*BMP*-*Wnt* circuit in a clinically aggressive stem cell-like subtype of HCC.

***miR-331-3p***

It has been reported that miR-331-3p is expressed abnormally in different tumor types, including gastric cancer, lung cancer, leukemia, and prostate cancer, and is associated with the proliferation and migration of cancers[134,135]. Chang *et al*[123] surveyed the expression of 840 miRNAs in HCC and identified miR-331-3p as one of the miRNAs that was significantly upregulated in HCC tissues. MiR-331-3p promoted the proliferation and metastasis of HCC cells *in vitro* and *in vivo* and was associated with an impaired prognosis[123]. PH domain and leucine-rich repeat protein phosphatase (PHLPP) was found to be a target of miR-331-3p. It was demonstrated that overexpression of PHLPP abrogated the influence of miR-331-3p on HCC cells, whereas silencing of *PHLPP* enhanced it. MiR-331-3p induced the phosphorylation of *AKT* and the subsequent epithelial-mesenchymal transition[123]. MiR-331-3p can be considered as a potential prognostic biomarker and therapeutic target.

**CONCLUSION**

HCC is a complex disease with multiple underlying pathogenic mechanisms caused by a variety of risk factors[18]. The lack of good molecular markers for HCC diagnosis and treatment assessment has posed a major challenge in health care[6,18]. The molecular profiling of genes provides powerful tools for gaining insight into the molecular mechanisms underlying hepatocarcinogenesis[136]. Knowledge obtained from such studies could be translated to develop new diagnostic, prognostic, and therapeutic targets for clinical intervention[18,137].

Sorafenib was the first compound shown to significantly improve the survival of patients with advanced HCC[10]. This multikinase inhibitor is active against Raf-1, VEGF receptor 2, B-Raf, the platelet-derived growth factor receptor and c-Kit as well as other receptor tyrosine and serine threonine kinases[16,138]. Preclinical models showed the significant anti-tumoral activity of sorafenib through reduced cell viability and induced apoptosis *in vitro* and *in vivo*[10]. The success of sorafenib is proof of the principle that molecular therapy plays an important role in the treatment of advanced HCC.

Further consideration should be given to developing more effective molecular diagnostic markers and targeted drug therapy. It is clear that genetic/epigenetic alterations are critical determinants of human hepatocellular cancer[9,118]. The progressive accumulation of genetic/epigenetic changes during the development of HCC provides a unique opportunity to use them as biomarkers in cancer detection[6,139]. However, not all aberrations may be equally important for the tumorigenic process[140]. It is unlikely that all epigenetic aberrations play a significant role in hepatocarcinogenesis. For example, some genetic/epigenetic changes may drive other events that contribute to the formation of a transformed phenotype, whereas others may be passenger events that accompany the transformation process[118].

By contrast, some compounds belonging to the group of demethylation agents have progressed to clinical trials in several human cancers[6,69]. One of the key limitations of these agents is that they do not act in a gene-specific manner; instead, they cause global hypomethylation of all genes with CpG sequences[69]. To date, the treatment outcome appears to be better in hematopoietic cancers than solid tumors[141]. The toxicity of nucleoside analogs due to the nonspecific hypomethylation of potential proto-oncogenes and retrotransposons has necessitated the development of other direct or indirect inhibitors of DNA methyltransferase (DNMT) activity, including procainamide, green tea polyphenols, epigallocatechin-3-gallate, and antisense oligodeoxynucleotides[142]. These compounds are less potent inhibitors of *DNMT* activity compared with 5-asa-dC-based compounds, but they present less toxicity and can potentially be used as an adjunct to nucleoside analog therapy[69,141,142].

Indeed miRNAs appears promising as diagnostic tools and miRNA-targeted therapy. However, the elucidation of downstream molecules is necessary to establish highly specific diagnostic and therapeutic approaches.

Although there is still a long way to go to reach the goal, the accumulation of knowledge regarding genetic and epigenetic factors is of key importance to elucidate the biological features of HCC and overcome this disease.

**REFERENCES**

1 **Tischoff I**, Tannapfe A. DNA methylation in hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1741-1748 [PMID: 18350605 DOI: 10.3748/wjg.14.1741]

2 **Siegel R**, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; **61**: 212-236 [PMID: 21685461 DOI: 10.3322/caac.20121]

3 **Gao Q**, Shi Y, Wang X, Zhou J, Qiu S, Fan J. Translational medicine in hepatocellular carcinoma. *Front Med* 2012; **6**: 122-133 [PMID: 22573220 DOI: 10.1007/s11684-012-0193-7]

4 **Bruix J**, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850 DOI: 10.1136/gutjnl-2013-306627]

5 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/s0140-6736(03)14964-1]

6 **Mínguez B**, Lachenmayer A. Diagnostic and prognostic molecular markers in hepatocellular carcinoma. *Dis Markers* 2011; **31**: 181-190 [PMID: 22045404 DOI: 10.3233/dma-2011-0841]

7 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

8 **Sanyal AJ**, Yoon SK, Lencioni R. The etiology of hepatocellular carcinoma and consequences for treatment. *Oncologist* 2010; **15 Suppl 4**: 14-22 [PMID: 21115577 DOI: 10.1634/theoncologist.2010-S4-14]

9 **Yates LR**, Campbell PJ. Evolution of the cancer genome. *Nat Rev Genet* 2012; **13**: 795-806 [PMID: 23044827 DOI: 10.1038/nrg3317]

10 **Lachenmayer A**, Alsinet C, Chang CY, Llovet JM. Molecular approaches to treatment of hepatocellular carcinoma. *Dig Liver Dis* 2010; **42 Suppl 3**: S264-S272 [PMID: 20547313 DOI: 10.1016/s1590-8658(10)60515-4]

11 **Kanda M**, Nomoto S, Okamura Y, Nishikawa Y, Sugimoto H, Kanazumi N, Takeda S, Nakao A. Detection of metallothionein 1G as a methylated tumor suppressor gene in human hepatocellular carcinoma using a novel method of double combination array analysis. *Int J Oncol* 2009; **35**: 477-483 [PMID: 19639168]

12 **Kanda M**, Nomoto S, Nishikawa Y, Sugimoto H, Kanazumi N, Takeda S, Nakao A. Correlations of the expression of vascular endothelial growth factor B and its isoforms in hepatocellular carcinoma with clinico-pathological parameters. *J Surg Oncol* 2008; **98**: 190-196 [PMID: 18537151 DOI: 10.1002/jso.21095]

13 **Giannelli G**, Rani B, Dituri F, Cao Y, Palasciano G. Moving towards personalised therapy in patients with hepatocellular carcinoma: the role of the microenvironment. *Gut* 2014; **63**: 1668-1676 [PMID: 25053718 DOI: 10.1136/gutjnl-2014-307323]

14 **Chan SL**, Yeo W. Targeted therapy of hepatocellular carcinoma: present and future. *J Gastroenterol Hepatol* 2012; **27**: 862-872 [PMID: 22369685 DOI: 10.1111/j.1440-1746.2012.07096.x]

15 **Miki D**, Ochi H, Hayes CN, Aikata H, Chayama K. Hepatocellular carcinoma: towards personalized medicine. *Cancer Sci* 2012; **103**: 846-850 [PMID: 22339805 DOI: 10.1111/j.1349-7006.2012.02242.x]

16 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

17 **Takai A**, Dang HT, Wang XW. Identification of drivers from cancer genome diversity in hepatocellular carcinoma. *Int J Mol Sci* 2014; **15**: 11142-11160 [PMID: 24955791 DOI: 10.3390/ijms150611142]

18 **Aravalli RN**, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. *Hepatology* 2008; **48**: 2047-2063 [PMID: 19003900 DOI: 10.1002/hep.22580]

19 **Hernandez-Gea V**, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* 2013; **144**: 512-527 [PMID: 23313965 DOI: 10.1053/j.gastro.2013.01.002]

20 **Sawan C**, Vaissière T, Murr R, Herceg Z. Epigenetic drivers and genetic passengers on the road to cancer. *Mutat Res* 2008; **642**: 1-13 [PMID: 18471836 DOI: 10.1016/j.mrfmmm.2008.03.002]

21 **Cho SB**, Lee WS, Park YL, Kim N, Oh HH, Kim MY, Oak CY, Chung CY, Park HC, Kim JS, Myung DS, Kim SH, Lee KH, Choi SK, Joo YE. Livin is associated with the invasive and oncogenic phenotypes of human hepatocellular carcinoma cells. *Hepatol Res* 2015; **45**: 448-457 [PMID: 24934632 DOI: 10.1111/hepr.12374]

22 **Du R**, Wu S, Lv X, Fang H, Wu S, Kang J. Overexpression of brachyury contributes to tumor metastasis by inducing epithelial-mesenchymal transition in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2014; **33**: 105 [PMID: 25499255 DOI: 10.1186/s13046-014-0105-6]

23 **Duan M**, Wang ZC, Wang XY, Shi JY, Yang LX, Ding ZB, Gao Q, Zhou J, Fan J. TREM-1, an Inflammatory Modulator, is Expressed in Hepatocellular Carcinoma Cells and Significantly Promotes Tumor Progression. *Ann Surg Oncol* 2014 Dec 3; Epub ahead of print [PMID: 25465376 DOI: 10.1245/s10434-014-4191-7]

24 **Fu Y**, Li J, Feng MX, Yang XM, Wang YH, Zhang YL, Qin W, Xia Q, Zhang ZG. Cytohesin-3 is upregulated in hepatocellular carcinoma and contributes to tumor growth and vascular invasion. *Int J Clin Exp Pathol* 2014; **7**: 2123-2132 [PMID: 24966920]

25 **Gai X**, Tu K, Lu Z, Zheng X. MRC2 expression correlates with TGFβ1 and survival in hepatocellular carcinoma. *Int J Mol Sci* 2014; **15**: 15011-15025 [PMID: 25162823 DOI: 10.3390/ijms150915011]

26 **Gao HJ**, Zhao MC, Zhang YJ, Zhou DS, Xu L, Li GB, Chen MS, Liu J. Monocarboxylate transporter 4 predicts poor prognosis in hepatocellular carcinoma and is associated with cell proliferation and migration. *J Cancer Res Clin Oncol* 2015; **141**: 1151-1162 [PMID: 25446815 DOI: 10.1007/s00432-014-1888-8]

27 **Gao Y**, Li Z, Guo X, Liu Y, Zhang K. DLX4 as a prognostic marker for hepatocellular carcinoma. *Neoplasma* 2014; **61**: 318-323 [PMID: 24824934]

28 **Hao C**, Zhu PX, Yang X, Han ZP, Jiang JH, Zong C, Zhang XG, Liu WT, Zhao QD, Fan TT, Zhang L, Wei LX. Overexpression of SIRT1 promotes metastasis through epithelial-mesenchymal transition in hepatocellular carcinoma. *BMC Cancer* 2014; **14**: 978 [PMID: 25522783 DOI: 10.1186/1471-2407-14-978]

29 **Hu ZY**, Yuan SX, Yang Y, Zhou WP, Jiang H. Pleomorphic adenoma gene 1 mediates the role of karyopherin alpha 2 and has prognostic significance in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2014; **33**: 61 [PMID: 25060425 DOI: 10.1186/s13046-014-0061-1]

30 **Huang X**, Wang X, Cheng C, Cai J, He S, Wang H, Liu F, Zhu C, Ding Z, Huang X, Zhang T, Zhang Y. Chaperonin containing TCP1, subunit 8 (CCT8) is upregulated in hepatocellular carcinoma and promotes HCC proliferation. *APMIS* 2014; **122**: 1070-1079 [PMID: 24862099 DOI: 10.1111/apm.12258]

31 **Jiang SS**, Weng DS, Wang QJ, Pan K, Zhang YJ, Li YQ, Li JJ, Zhao JJ, He J, Lv L, Pan QZ, Xia JC. Galectin-3 is associated with a poor prognosis in primary hepatocellular carcinoma. *J Transl Med* 2014; **12**: 273 [PMID: 25260879 DOI: 10.1186/s12967-014-0273-3]

32 **Jin S**, Wang K, Xu K, Xu J, Sun J, Chu Z, Lin D, Koeffler PH, Wang J, Yin D. Oncogenic function and prognostic significance of protein tyrosine phosphatase PRL-1 in hepatocellular carcinoma. *Oncotarget* 2014; **5**: 3685-3696 [PMID: 25003523]

33 **Li C**, Wang J, Zhang H, Zhu M, Chen F, Hu Y, Liu H, Zhu H. Interferon-stimulated gene 15 (ISG15) is a trigger for tumorigenesis and metastasis of hepatocellular carcinoma. *Oncotarget* 2014; **5**: 8429-8441 [PMID: 25238261]

34 **Li S**, Ma W, Fei T, Lou Q, Zhang Y, Cui X, Qin X, Zhang J, Liu G, Dong Z, Ma Y, Song Z, Hu Y. Upregulation of heat shock factor 1 transcription activity is associated with hepatocellular carcinoma progression. *Mol Med Rep* 2014; **10**: 2313-2321 [PMID: 25199534 DOI: 10.3892/mmr.2014.2547]

35 **Liu Q**, Tu K, Zhang H, Zheng X, Yao Y, Liu Q. TPX2 as a novel prognostic biomarker for hepatocellular carcinoma. *Hepatol Res* 2015; **45**: 906-918 [PMID: 25263743 DOI: 10.1111/hepr.12428]

36 **Ruan J**, Zheng H, Fu W, Zhao P, Su N, Luo R. Increased expression of cathepsin L: a novel independent prognostic marker of worse outcome in hepatocellular carcinoma patients. *PLoS One* 2014; **9**: e112136 [PMID: 25384089 DOI: 10.1371/journal.pone.0112136]

37 **Sun W**, Su Q, Cao X, Shang B, Chen A, Yin H, Liu B. High expression of polo-like kinase 1 is associated with early development of hepatocellular carcinoma. *Int J Genomics* 2014; **2014**: 312130 [PMID: 25019081 DOI: 10.1155/2014/312130]

38 **Wei T**, Zhang LN, Lv Y, Ma XY, Zhi L, Liu C, Ma F, Zhang XF. Overexpression of platelet-derived growth factor receptor alpha promotes tumor progression and indicates poor prognosis in hepatocellular carcinoma. *Oncotarget* 2014; **5**: 10307-10317 [PMID: 25333264]

39 **Xue TC**, Ge NL, Zhang L, Cui JF, Chen RX, You Y, Ye SL, Ren ZG. Goosecoid promotes the metastasis of hepatocellular carcinoma by modulating the epithelial-mesenchymal transition. *PLoS One* 2014; **9**: e109695 [PMID: 25343336 DOI: 10.1371/journal.pone.0109695]

40 **Zhang K**, Chen J, Chen D, Huang J, Feng B, Han S, Chen Y, Song H, De W, Zhu Z, Wang R, Chen L. Aurora-A promotes chemoresistance in hepatocelluar carcinoma by targeting NF-kappaB/microRNA-21/PTEN signaling pathway. *Oncotarget* 2014; **5**: 12916-12935 [PMID: 25428915]

41 **Cannito S**, Turato C, Paternostro C, Biasiolo A, Colombatto S, Cambieri I, Quarta S, Novo E, Morello E, Villano G, Fasolato S, Musso T, David E, Tusa I, Rovida E, Autelli R, Smedile A, Cillo U, Pontisso P, Parola M. Hypoxia up-regulates SERPINB3 through HIF-2α in human liver cancer cells. *Oncotarget* 2015; **6**: 2206-2221 [PMID: 25544768]

42 **Chang R**, Wei L, Lu Y, Cui X, Lu C, Liu L, Jiang D, Xiong Y, Wang G, Wan C, Qian H. Upregulated expression of ubiquitin-conjugating enzyme E2Q1 (UBE2Q1) is associated with enhanced cell proliferation and poor prognosis in human hapatocellular carcinoma. *J Mol Histol* 2015; **46**: 45-56 [PMID: 25311764 DOI: 10.1007/s10735-014-9596-x]

43 **Chang TS**, Wu YC, Chi CC, Su WC, Chang PJ, Lee KF, Tung TH, Wang J, Liu JJ, Tung SY, Kuo LM, Ho HN, Ling TY, Huang YH. Activation of IL6/IGFIR confers poor prognosis of HBV-related hepatocellular carcinoma through induction of OCT4/NANOG expression. *Clin Cancer Res* 2015; **21**: 201-210 [PMID: 25564572 DOI: 10.1158/1078-0432.ccr-13-3274]

44 **Dai T**, Zhang D, Cai M, Wang C, Wu Z, Ying Z, Wu J, Li M, Xie D, Li J, Song L. Golgi phosphoprotein 3 (GOLPH3) promotes hepatocellular carcinoma cell aggressiveness by activating the NF-κB pathway. *J Pathol* 2015; **235**: 490-501 [PMID: 25385148 DOI: 10.1002/path.4479]

45 **Hou KZ**, Fu ZQ, Gong H. Chemokine ligand 20 enhances progression of hepatocellular carcinoma via epithelial-mesenchymal transition. *World J Gastroenterol* 2015; **21**: 475-483 [PMID: 25593462 DOI: 10.3748/wjg.v21.i2.475]

46 **Liu WR**, Tian MX, Yang LX, Lin YL, Jin L, Ding ZB, Shen YH, Peng YF, Gao DM, Zhou J, Qiu SJ, Dai Z, He R, Fan J, Shi YH. PKM2 promotes metastasis by recruiting myeloid-derived suppressor cells and indicates poor prognosis for hepatocellular carcinoma. *Oncotarget* 2015; **6**: 846-861 [PMID: 25514599]

47 **Yao Y**, Dou C, Lu Z, Zheng X, Liu Q. MACC1 suppresses cell apoptosis in hepatocellular carcinoma by targeting the HGF/c-MET/AKT pathway. *Cell Physiol Biochem* 2015; **35**: 983-996 [PMID: 25660117 DOI: 10.1159/000369754]

48 **Zhang XF**, Pan QZ, Pan K, Weng DS, Wang QJ, Zhao JJ, He J, Liu Q, Wang DD, Jiang SS, Zheng HX, Lv L, Chen CL, Zhang HX, Xia JC. Expression and prognostic role of ubiquitination factor E4B in primary hepatocellular carcinoma. *Mol Carcinog* 2015; : [PMID: 25557723 DOI: 10.1002/mc.22259]

49 **Zhao X**, Parpart S, Takai A, Roessler S, Budhu A, Yu Z, Blank M, Zhang YE, Jia HL, Ye QH, Qin LX, Tang ZY, Thorgeirsson SS, Wang XW. Integrative genomics identifies YY1AP1 as an oncogenic driver in EpCAM(+) AFP(+) hepatocellular carcinoma. *Oncogene* 2015 Jan 19; Epub ahead of print [PMID: 25597408 DOI: 10.1038/onc.2014.438]

50 **Takami H**, Kanda M, Oya H, Hibino S, Sugimoto H, Suenaga M, Yamada S, Nishikawa Y, Asai M, Fujii T, Nomoto S, Kodera Y. Evaluation of MAGE-D4 expression in hepatocellular carcinoma in Japanese patients. *J Surg Oncol* 2013; **108**: 557-562 [PMID: 24068544 DOI: 10.1002/jso.23440]

51 **Hashimoto R**, Kanda M, Takami H, Shimizu D, Oya H, Hibino S, Okamura Y, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Nomoto S, Fujiwara M, Kodera Y. Aberrant expression of melanoma-associated antigen-D2 serves as a prognostic indicator of hepatocellular carcinoma outcome following curative hepatectomy. *Oncol Lett* 2015; **9**: 1201-1206 [PMID: 25663882 DOI: 10.3892/ol.2014.2823]

52 **Berasain C**, Castillo J, Perugorria MJ, Latasa MU, Prieto J, Avila MA. Inflammation and liver cancer: new molecular links . *Ann N Y Acad Sci* 2009; **1155**: 206-221 [PMID: 19250206 DOI: 10.1111/j.1749-6632.2009.03704.x]

53 **Hösel M**, Quasdorff M, Wiegmann K, Webb D, Zedler U, Broxtermann M, Tedjokusumo R, Esser K, Arzberger S, Kirschning CJ, Langenkamp A, Falk C, Büning H, Rose-John S, Protzer U. Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* 2009; **50**: 1773-1782 [PMID: 19937696 DOI: 10.1002/hep.23226]

54 **Forte G**, Minieri M, Cossa P, Antenucci D, Sala M, Gnocchi V, Fiaccavento R, Carotenuto F, De Vito P, Baldini PM, Prat M, Di Nardo P. Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. *Stem Cells* 2006; **24**: 23-33 [PMID: 16100005 DOI: 10.1634/stemcells.2004-0176]

55 **Ma PC**, Maulik G, Christensen J, Salgia R. c-Met: structure, functions and potential for therapeutic inhibition. *Cancer Metastasis Rev* 2003; **22**: 309-325 [PMID: 12884908]

56 **Michan S**, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J* 2007; **404**: 1-13 [PMID: 17447894 DOI: 10.1042/bj20070140]

57 **Blander G**, Guarente L. The Sir2 family of protein deacetylases. *Annu Rev Biochem* 2004; **73**: 417-435 [PMID: 15189148 DOI: 10.1146/annurev.biochem.73.011303.073651]

58 **Bae HJ**, Noh JH, Kim JK, Eun JW, Jung KH, Kim MG, Chang YG, Shen Q, Kim SJ, Park WS, Lee JY, Nam SW. MicroRNA-29c functions as a tumor suppressor by direct targeting oncogenic SIRT1 in hepatocellular carcinoma. *Oncogene* 2014; **33**: 2557-2567 [PMID: 23728341 DOI: 10.1038/onc.2013.216]

59 **Zhang ZY**, Hong D, Nam SH, Kim JM, Paik YH, Joh JW, Kwon CH, Park JB, Choi GS, Jang KY, Park CK, Kim SJ. SIRT1 regulates oncogenesis via a mutant p53-dependent pathway in hepatocellular carcinoma. *J Hepatol* 2015; **62**: 121-130 [PMID: 25131770 DOI: 10.1016/j.jhep.2014.08.007]

60 **Chang H**, Wang J, Tian Y, Xu J, Gou X, Cheng J. The TPX2 gene is a promising diagnostic and therapeutic target for cervical cancer. *Oncol Rep* 2012; **27**: 1353-1359 [PMID: 22307108 DOI: 10.3892/or.2012.1668]

61 **Wittmann T**, Wilm M, Karsenti E, Vernos I. TPX2, A novel xenopus MAP involved in spindle pole organization. *J Cell Biol* 2000; **149**: 1405-1418 [PMID: 10871281]

62 **Huang Y**, Guo W, Kan H. TPX2 is a prognostic marker and contributes to growth and metastasis of human hepatocellular carcinoma. *Int J Mol Sci* 2014; **15**: 18148-18161 [PMID: 25302620 DOI: 10.3390/ijms151018148]

63 **Galli R**, Starace D, Busà R, Angelini DF, Paone A, De Cesaris P, Filippini A, Sette C, Battistini L, Ziparo E, Riccioli A. TLR stimulation of prostate tumor cells induces chemokine-mediated recruitment of specific immune cell types. *J Immunol* 2010; **184**: 6658-6669 [PMID: 20483744 DOI: 10.4049/jimmunol.0902401]

64 **Dower K**, Ellis DK, Saraf K, Jelinsky SA, Lin LL. Innate immune responses to TREM-1 activation: overlap, divergence, and positive and negative cross-talk with bacterial lipopolysaccharide. *J Immunol* 2008; **180**: 3520-3534 [PMID: 18292579]

65 **Chang CC**, Campoli M, Luo W, Zhao W, Zaenker KS, Ferrone S. Immunotherapy of melanoma targeting human high molecular weight melanoma-associated antigen: potential role of nonimmunological mechanisms. *Ann N Y Acad Sci* 2004; **1028**: 340-350 [PMID: 15650259 DOI: 10.1196/annals.1322.040]

66 **Chomez P**, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. An overview of the MAGE gene family with the identification of all human members of the family. *Cancer Res* 2001; **61**: 5544-5551 [PMID: 11454705]

67 **Sang M**, Wang L, Ding C, Zhou X, Wang B, Wang L, Lian Y, Shan B. Melanoma-associated antigen genes - an update. *Cancer Lett* 2011; **302**: 85-90 [PMID: 21093980 DOI: 10.1016/j.canlet.2010.10.021]

68 **Kanda M**, Nomoto S, Oya H, Takami H, Shimizu D, Hibino S, Hashimoto R, Kobayashi D, Tanaka C, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Fujiwara M, Kodera Y. The Expression of Melanoma-Associated Antigen D2 Both in Surgically Resected and Serum Samples Serves as Clinically Relevant Biomarker of Gastric Cancer Progression. *Ann Surg Oncol* 2015; : [PMID: 25743330 DOI: 10.1245/s10434-015-4457-8]

69 **Boland CR**, Shin SK, Goel A. Promoter methylation in the genesis of gastrointestinal cancer. *Yonsei Med J* 2009; **50**: 309-321 [PMID: 19568590 DOI: 10.3349/ymj.2009.50.3.309]

70 **Villanueva A**, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007; **27**: 55-76 [PMID: 17295177 DOI: 10.1055/s-2006-960171]

71 Bauer R, Valletta D, Bauer K, Thasler WE, Hartmann A, Muller M, Reichert TE, Hellerbrand C. Downregulation of P-cadherin expression in hepatocellular carcinoma induces tumorigenicity. *Int J Clin Exp Pathol* 2014; **7**: 6125-6132 [PMID: 25337260]

72 **Cai Z**, Zeng Y, Xu B, Gao Y, Wang S, Zeng J, Chen L, Huang A, Liu X, Liu J. Galectin-4 serves as a prognostic biomarker for the early recurrence / metastasis of hepatocellular carcinoma. *Cancer Sci* 2014; **105**: 1510-1517 [PMID: 25230111 DOI: 10.1111/cas.12536]

73 **Dai B**, Ruan B, Wu J, Wang J, Shang R, Sun W, Li X, Dou K, Wang D, Li Y. Insulin-like growth factor binding protein-1 inhibits cancer cell invasion and is associated with poor prognosis in hepatocellular carcinoma. *Int J Clin Exp Pathol* 2014; **7**: 5645-5654 [PMID: 25337205]

74 **Hu B**, Xiong Y, Ni R, Wei L, Jiang D, Wang G, Wu D, Xu T, Zhao F, Zhu M, Wan C. The downregulation of ErbB3 binding protein 1 (EBP1) is associated with poor prognosis and enhanced cell proliferation in hepatocellular carcinoma. *Mol Cell Biochem* 2014; **396**: 175-185 [PMID: 25081333 DOI: 10.1007/s11010-014-2153-9]

75 **Qi X**, Ng KT, Lian QZ, Liu XB, Li CX, Geng W, Ling CC, Ma YY, Yeung WH, Tu WW, Fan ST, Lo CM, Man K. Clinical significance and therapeutic value of glutathione peroxidase 3 (GPx3) in hepatocellular carcinoma. *Oncotarget* 2014; **5**: 11103-11120 [PMID: 25333265]

76 **Tu K**, Dou C, Zheng X, Li C, Yang W, Yao Y, Liu Q. Fibulin-5 inhibits hepatocellular carcinoma cell migration and invasion by down-regulating matrix metalloproteinase-7 expression. *BMC Cancer* 2014; **14**: 938 [PMID: 25494879 DOI: 10.1186/1471-2407-14-938]

77 **Tu K**, Yang W, Li C, Zheng X, Lu Z, Guo C, Yao Y, Liu Q. Fbxw7 is an independent prognostic marker and induces apoptosis and growth arrest by regulating YAP abundance in hepatocellular carcinoma. *Mol Cancer* 2014; **13**: 110 [PMID: 24884509 DOI: 10.1186/1476-4598-13-110]

78 **Zhang Z**, Liang X, Gao L, Ma H, Liu X, Pan Y, Yan W, Shan H, Wang Z, Chen YH, Ma C. TIPE1 induces apoptosis by negatively regulating Rac1 activation in hepatocellular carcinoma cells. *Oncogene* 2015; **34**: 2566-2574 [PMID: 25043299 DOI: 10.1038/onc.2014.208]

79 **Li W**, Liang Y, Yang B, Sun H, Wu W. Downregulation of ARNT2 promotes tumor growth and predicts poor prognosis in human hepatocellular carcinoma. *J Gastroenterol Hepatol* 2015; **30**: 1085-1093 [PMID: 25611915 DOI: 10.1111/jgh.12905]

80 **Kanda M**, Sugimoto H, Nomoto S, Oya H, Hibino S, Shimizu D, Takami H, Hashimoto R, Okamura Y, Yamada S, Fujii T, Nakayama G, Koike M, Fujiwara M, Kodera Y. B‑cell translocation gene 1 serves as a novel prognostic indicator of hepatocellular carcinoma. *Int J Oncol* 2015; **46**: 641-648 [PMID: 25405901 DOI: 10.3892/ijo.2014.2762]

81 **Shi S**, Yoon DY, Hodge-Bell KC, Bebenek IG, Whitekus MJ, Zhang R, Cochran AJ, Huerta-Yepez S, Yim SH, Gonzalez FJ, Jaiswal AK, Hankinson O. The aryl hydrocarbon receptor nuclear translocator (Arnt) is required for tumor initiation by benzo[a]pyrene. *Carcinogenesis* 2009; **30**: 1957-1961 [PMID: 19755658 DOI: 10.1093/carcin/bgp201]

82 **Reyes H**, Reisz-Porszasz S, Hankinson O. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* 1992; **256**: 1193-1195 [PMID: 1317062 DOI: 10.1126/science.256.5060.1193]

83 **Prévôt D**, Voeltzel T, Birot AM, Morel AP, Rostan MC, Magaud JP, Corbo L. The leukemia-associated protein Btg1 and the p53-regulated protein Btg2 interact with the homeoprotein Hoxb9 and enhance its transcriptional activation. *J Biol Chem* 2000; **275**: 147-153 [PMID: 10617598 DOI: 10.1074/jbc.275.1.147]

84 **Kanda M**, Oya H, Nomoto S, Takami H, Shimizu D, Hashimoto R, Sueoka S, Kobayashi D, Tanaka C, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Fujiwara M, Kodera Y. Diversity of clinical implication of B-cell translocation gene 1 expression by histopathologic and anatomic subtypes of gastric cancer. *Dig Dis Sci* 2015; **60**: 1256-1264 [PMID: 25487193 DOI: 10.1007/s10620-014-3477-8]

85 **Zhao Y**, Gou WF, Chen S, Takano Y, Xiu YL, Zheng HC. BTG1 expression correlates with the pathogenesis and progression of ovarian carcinomas. *Int J Mol Sci* 2013; **14**: 19670-19680 [PMID: 24084718 DOI: 10.3390/ijms141019670]

86 **Li YG**, Ji DF, Zhong S, Shi LG, Hu GY, Chen S. Saponins from Panax japonicus protect against alcohol-induced hepatic injury in mice by up-regulating the expression of GPX3, SOD1 and SOD3. *Alcohol Alcohol* 2010; **45**: 320-331 [PMID: 20554696 DOI: 10.1093/alcalc/agq034]

87 **Timpl R**, Sasaki T, Kostka G, Chu ML. Fibulins: a versatile family of extracellular matrix proteins. *Nat Rev Mol Cell Biol* 2003; **4**: 479-489 [PMID: 12778127 DOI: 10.1038/nrm1130]

88 **Nakamura T**, Lozano PR, Ikeda Y, Iwanaga Y, Hinek A, Minamisawa S, Cheng CF, Kobuke K, Dalton N, Takada Y, Tashiro K, Ross Jr J, Honjo T, Chien KR. Fibulin-5/DANCE is essential for elastogenesis in vivo. *Nature* 2002; **415**: 171-175 [PMID: 11805835 DOI: 10.1038/415171a]

89 **Northington GM**. Fibulin-5: two for the price of one maintaining pelvic support. *J Clin Invest* 2011; **121**: 1688-1691 [PMID: 21519147 DOI: 10.1172/jci57438]

90 **Khare S**, Zhang Q, Ibdah JA. Epigenetics of hepatocellular carcinoma: role of microRNA. *World J Gastroenterol* 2013; **19**: 5439-5445 [PMID: 24023486 DOI: 10.3748/wjg.v19.i33.5439]

91 **Sceusi EL**, Loose DS, Wray CJ. Clinical implications of DNA methylation in hepatocellular carcinoma. *HPB (Oxford)* 2011; **13**: 369-376 [PMID: 21609368 DOI: 10.1111/j.1477-2574.2011.00303.x]

92 **Kanda M**, Shimizu D, Nomoto S, Hibino S, Oya H, Takami H, Kobayashi D, Yamada S, Inokawa Y, Tanaka C, Fujii T, Sugimoto H, Koike M, Fujiwara M, Kodera Y. Clinical significance of expression and epigenetic profiling of TUSC1 in gastric cancer. *J Surg Oncol* 2014; **110**: 136-144 [PMID: 24700496 DOI: 10.1002/jso.23614]

93 **Kanda M**, Nomoto S, Oya H, Hashimoto R, Takami H, Shimizu D, Sonohara F, Kobayashi D, Tanaka C, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Murotani K, Fujiwara M, Kodera Y. Decreased expression of prenyl diphosphate synthase subunit 2 correlates with reduced survival of patients with gastric cancer. *J Exp Clin Cancer Res* 2014; **33**: 88 [PMID: 25330808 DOI: 10.1186/preaccept-8549609481376418]

94 **Arzumanyan A**, Reis HM, Feitelson MA. Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. *Nat Rev Cancer* 2013; **13**: 123-135 [PMID: 23344543 DOI: 10.1038/nrc3449]

95 **Kanda M**, Shimizu D, Nomoto S, Takami H, Hibino S, Oya H, Hashimoto R, Suenaga M, Inokawa Y, Kobayashi D, Tanaka C, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Fujiwara M, Kodera Y. Prognostic impact of expression and methylation status of DENN/MADD domain-containing protein 2D in gastric cancer. *Gastric Cancer* 2015; **18**: 288-296 [PMID: 24695972 DOI: 10.1007/s10120-014-0372-0]

96 **Kanda M**, Nomoto S, Okamura Y, Hayashi M, Hishida M, Fujii T, Nishikawa Y, Sugimoto H, Takeda S, Nakao A. Promoter hypermethylation of fibulin 1 gene is associated with tumor progression in hepatocellular carcinoma. *Mol Carcinog* 2011; **50**: 571-579 [PMID: 21268132 DOI: 10.1002/mc.20735]

97 **Ding ZY**, Jin GN, Wang W, Chen WX, Wu YH, Ai X, Chen L, Zhang WG, Liang HF, Laurence A, Zhang MZ, Datta PK, Zhang B, Chen XP. Reduced expression of transcriptional intermediary factor 1 gamma promotes metastasis and indicates poor prognosis of hepatocellular carcinoma. *Hepatology* 2014; **60**: 1620-1636 [PMID: 24954480 DOI: 10.1002/hep.27273]

98 **Fu Y**, Feng MX, Yu J, Ma MZ, Liu XJ, Li J, Yang XM, Wang YH, Zhang YL, Ao JP, Xue F, Qin W, Gu J, Xia Q, Zhang ZG. DNA methylation-mediated silencing of matricellular protein dermatopontin promotes hepatocellular carcinoma metastasis by α3β1 integrin-Rho GTPase signaling. *Oncotarget* 2014; **5**: 6701-6715 [PMID: 25149533]

99 **Jiang L**, Yang YD, Fu L, Xu W, Liu D, Liang Q, Zhang X, Xu L, Guan XY, Wu B, Sung JJ, Yu J. CLDN3 inhibits cancer aggressiveness via Wnt-EMT signaling and is a potential prognostic biomarker for hepatocellular carcinoma. *Oncotarget* 2014; **5**: 7663-7676 [PMID: 25277196]

100 **Kanda M**, Nomoto S, Oya H, Takami H, Hibino S, Hishida M, Suenaga M, Yamada S, Inokawa Y, Nishikawa Y, Asai M, Fujii T, Sugimoto H, Kodera Y. Downregulation of DENND2D by promoter hypermethylation is associated with early recurrence of hepatocellular carcinoma. *Int J Oncol* 2014; **44**: 44-52 [PMID: 24189587 DOI: 10.3892/ijo.2013.2165]

101 **Kanda M**, Sugimoto H, Nomoto S, Oya H, Shimizu D, Takami H, Hashimoto R, Sonohara F, Okamura Y, Yamada S, Fujii T, Nakayama G, Koike M, Fujiwara M, Kodera Y. Clinical utility of PDSS2 expression to stratify patients at risk for recurrence of hepatocellular carcinoma. *Int J Oncol* 2014; **45**: 2005-2012 [PMID: 25189544 DOI: 10.3892/ijo.2014.2637]

102 **Zhuo H**, Tang J, Lin Z, Jiang R, Zhang X, Ji J, Wang P, Sun B. The aberrant expression of MEG3 regulated by UHRF1 predicts the prognosis of hepatocellular carcinoma. *Mol Carcinog* 2015; : [PMID: 25641194 DOI: 10.1002/mc.22270]

103 **Nomoto S**, Kanda M, Okamura Y, Nishikawa Y, Qiyong L, Fujii T, Sugimoto H, Takeda S, Nakao A. Epidermal growth factor-containing fibulin-like extracellular matrix protein 1, EFEMP1, a novel tumor-suppressor gene detected in hepatocellular carcinoma using double combination array analysis. *Ann Surg Oncol* 2010; **17**: 923-932 [PMID: 19898900 DOI: 10.1245/s10434-009-0790-0]

104 **Okamura Y**, Nomoto S, Kanda M, Hayashi M, Nishikawa Y, Fujii T, Sugimoto H, Takeda S, Nakao A. Reduced expression of reelin (RELN) gene is associated with high recurrence rate of hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 572-579 [PMID: 20734148 DOI: 10.1245/s10434-010-1273-z]

105 **Oya H**, Kanda M, Sugimoto H, Shimizu D, Takami H, Hibino S, Hashimoto R, Okamura Y, Yamada S, Fujii T, Nakayama G, Koike M, Nomoto S, Fujiwara M, Kodera Y. Dihydropyrimidinase-like 3 is a putative hepatocellular carcinoma tumor suppressor. *J Gastroenterol* 2015; **50**: 590-600 [PMID: 25173447 DOI: 10.1007/s00535-014-0993-4]

106 **Shimizu D**, Kanda M, Nomoto S, Oya H, Takami H, Hibino S, Suenaga M, Inokawa Y, Hishida M, Takano N, Nishikawa Y, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Fujiwara M, Kodera Y. Identification of intragenic methylation in the TUSC1 gene as a novel prognostic marker of hepatocellular carcinoma. *Oncol Rep* 2014; **31**: 1305-1313 [PMID: 24366000 DOI: 10.3892/or.2013.2939]

107 **Hatakeyama S**. TRIM proteins and cancer. *Nat Rev Cancer* 2011; **11**: 792-804 [PMID: 21979307 DOI: 10.1038/nrc3139]

108 **Bai X**, Kim J, Yang Z, Jurynec MJ, Akie TE, Lee J, LeBlanc J, Sessa A, Jiang H, DiBiase A, Zhou Y, Grunwald DJ, Lin S, Cantor AB, Orkin SH, Zon LI. TIF1gamma controls erythroid cell fate by regulating transcription elongation. *Cell* 2010; **142**: 133-143 [PMID: 20603019 DOI: 10.1016/j.cell.2010.05.028]

109 **Blasco H**, Bernard-Marissal N, Vourc'h P, Guettard YO, Sunyach C, Augereau O, Khederchah J, Mouzat K, Antar C, Gordon PH, Veyrat-Durebex C, Besson G, Andersen PM, Salachas F, Meininger V, Camu W, Pettmann B, Andres CR, Corcia P. A rare motor neuron deleterious missense mutation in the DPYSL3 (CRMP4) gene is associated with ALS. *Hum Mutat* 2013; **34**: 953-960 [PMID: 23568759 DOI: 10.1002/humu.22329]

110 **Kanda M**, Nomoto S, Oya H, Shimizu D, Takami H, Hibino S, Hashimoto R, Kobayashi D, Tanaka C, Yamada S, Fujii T, Nakayama G, Sugimoto H, Koike M, Fujiwara M, Kodera Y. Dihydropyrimidinase-like 3 facilitates malignant behavior of gastric cancer. *J Exp Clin Cancer Res* 2014; **33**: 66 [PMID: 25096402 DOI: 10.1186/preaccept-2175168251300157]

111 **Kawahara T**, Hotta N, Ozawa Y, Kato S, Kano K, Yokoyama Y, Nagino M, Takahashi T, Yanagisawa K. Quantitative proteomic profiling identifies DPYSL3 as pancreatic ductal adenocarcinoma-associated molecule that regulates cell adhesion and migration by stabilization of focal adhesion complex. *PLoS One* 2013; **8**: e79654 [PMID: 24339867 DOI: 10.1371/journal.pone.0079654]

112 **Gao X**, Pang J, Li LY, Liu WP, Di JM, Sun QP, Fang YQ, Liu XP, Pu XY, He D, Li MT, Su ZL, Li BY. Expression profiling identifies new function of collapsin response mediator protein 4 as a metastasis-suppressor in prostate cancer. *Oncogene* 2010; **29**: 4555-4566 [PMID: 20543870 DOI: 10.1038/onc.2010.213]

113 **Okamoto O**, Hozumi K, Katagiri F, Takahashi N, Sumiyoshi H, Matsuo N, Yoshioka H, Nomizu M, Fujiwara S. Dermatopontin promotes epidermal keratinocyte adhesion via alpha3beta1 integrin and a proteoglycan receptor. *Biochemistry* 2010; **49**: 147-155 [PMID: 19928997 DOI: 10.1021/bi901066f]

114 **Okamoto O**, Fujiwara S, Abe M, Sato Y. Dermatopontin interacts with transforming growth factor beta and enhances its biological activity. *Biochem J* 1999; **337 ( Pt 3)**: 537-541 [PMID: 9895299]

115 **Saiki R**, Nagata A, Kainou T, Matsuda H, Kawamukai M. Characterization of solanesyl and decaprenyl diphosphate synthases in mice and humans. *FEBS J* 2005; **272**: 5606-5622 [PMID: 16262699 DOI: 10.1111/j.1742-4658.2005.04956.x]

116 **Fung JM**, Smith R, Brown MA, Lau SH, Xie D, Lau GK, Guan XY. Identification and characterization of a novel melanoma tumor suppressor gene on human chromosome 6q21. *Clin Cancer Res* 2009; **15**: 797-803 [PMID: 19188149 DOI: 10.1158/1078-0432.ccr-08-1472]

117 **Gupta P**, Cairns MJ, Saksena NK. Regulation of gene expression by microRNA in HCV infection and HCV-mediated hepatocellular carcinoma. *Virol J* 2014; **11**: 64 [PMID: 24690114 DOI: 10.1186/1743-422x-11-64]

118 **Pogribny IP**, Rusyn I. Role of epigenetic aberrations in the development and progression of human hepatocellular carcinoma. *Cancer Lett* 2014; **342**: 223-230 [PMID: 22306342 DOI: 10.1016/j.canlet.2012.01.038]

119 **Shibata C**, Otsuka M, Kishikawa T, Ohno M, Yoshikawa T, Takata A, Koike K. Diagnostic and therapeutic application of noncoding RNAs for hepatocellular carcinoma. *World J Hepatol* 2015; **7**: 1-6 [PMID: 25624991 DOI: 10.4254/wjh.v7.i1.1]

120 **Hung CH**, Chiu YC, Chen CH, Hu TH. MicroRNAs in hepatocellular carcinoma: carcinogenesis, progression, and therapeutic target. *Biomed Res Int* 2014; **2014**: 486407 [PMID: 24800233 DOI: 10.1155/2014/486407]

121 **Schütte K**, Schulz C, Link A, Malfertheiner P. Current biomarkers for hepatocellular carcinoma: Surveillance, diagnosis and prediction of prognosis. *World J Hepatol* 2015; **7**: 139-149 [PMID: 25729470 DOI: 10.4254/wjh.v7.i2.139]

122 **Cai L**, Cai X. Up-regulation of miR-9 expression predicate advanced clinicopathological features and poor prognosis in patients with hepatocellular carcinoma. *Diagn Pathol* 2014; **9**: 1000 [PMID: 25552204 DOI: 10.1186/s13000-014-0228-2]

123 **Chang RM**, Yang H, Fang F, Xu JF, Yang LY. MicroRNA-331-3p promotes proliferation and metastasis of hepatocellular carcinoma by targeting PH domain and leucine-rich repeat protein phosphatase. *Hepatology* 2014; **60**: 1251-1263 [PMID: 24825302 DOI: 10.1002/hep.27221]

124 **Li B**, Huang P, Qiu J, Liao Y, Hong J, Yuan Y. MicroRNA-130a is down-regulated in hepatocellular carcinoma and associates with poor prognosis. *Med Oncol* 2014; **31**: 230 [PMID: 25218269 DOI: 10.1007/s12032-014-0230-2]

125 **Li BK**, Huang PZ, Qiu JL, Liao YD, Hong J, Yuan YF. Upregulation of microRNA-106b is associated with poor prognosis in hepatocellular carcinoma. *Diagn Pathol* 2014; **9**: 226 [PMID: 25466449 DOI: 10.1186/s13000-014-0226-4]

126 **Wang WY**, Zhang HF, Wang L, Ma YP, Gao F, Zhang SJ, Wang LC. High expression of microRNA-130b correlates with poor prognosis of patients with hepatocellular carcinoma. *Diagn Pathol* 2014; **9**: 160 [PMID: 25123453 DOI: 10.1186/s13000-014-0160-5]

127 **Xing TJ**, Jiang DF, Huang JX, Xu ZL. Expression and clinical significance of miR-122 and miR-29 in hepatitis B virus-related liver disease. *Genet Mol Res* 2014; **13**: 7912-7918 [PMID: 25299106 DOI: 10.4238/2014.September.29.4]

128 **Yu L**, Zhang J, Guo X, Li Z, Zhang P. MicroRNA-224 upregulation and AKT activation synergistically predict poor prognosis in patients with hepatocellular carcinoma. *Cancer Epidemiol* 2014; **38**: 408-413 [PMID: 24923856 DOI: 10.1016/j.canep.2014.05.001]

129 **He X**, Li J, Guo W, Liu W, Yu J, Song W, Dong L, Wang F, Yu S, Zheng Y, Chen S, Kong Y, Liu C. Targeting the microRNA-21/AP1 axis by 5-fluorouracil and pirarubicin in human hepatocellular carcinoma. *Oncotarget* 2015; **6**: 2302-2314 [PMID: 25544773]

130 **Huang B**, Li H, Huang L, Luo C, Zhang Y. Clinical significance of microRNA 138 and cyclin D3 in hepatocellular carcinoma. *J Surg Res* 2015; **193**: 718-723 [PMID: 25439221 DOI: 10.1016/j.jss.2014.03.076]

131 **Li L**, Liu Y, Guo Y, Liu B, Zhao Y, Li P, Song F, Zheng H, Yu J, Song T, Niu R, Li Q, Wang XW, Zhang W, Chen K. Regulatory MiR-148a-ACVR1/BMP circuit defines a cancer stem cell-like aggressive subtype of hepatocellular carcinoma. *Hepatology* 2015; **61**: 574-584 [PMID: 25271001 DOI: 10.1002/hep.27543]

132 **Zhuang L**, Xu L, Wang P, Meng Z. Serum miR-128-2 serves as a prognostic marker for patients with hepatocellular carcinoma. *PLoS One* 2015; **10**: e0117274 [PMID: 25642945 DOI: 10.1371/journal.pone.0117274]

133 **Shore EM**, Xu M, Feldman GJ, Fenstermacher DA, Cho TJ, Choi IH, Connor JM, Delai P, Glaser DL, LeMerrer M, Morhart R, Rogers JG, Smith R, Triffitt JT, Urtizberea JA, Zasloff M, Brown MA, Kaplan FS. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat Genet* 2006; **38**: 525-527 [PMID: 16642017 DOI: 10.1038/ng1783]

134 **Guo X**, Guo L, Ji J, Zhang J, Zhang J, Chen X, Cai Q, Li J, Gu Q, Liu B, Zhu Z, Yu Y. miRNA-331-3p directly targets E2F1 and induces growth arrest in human gastric cancer. *Biochem Biophys Res Commun* 2010; **398**: 1-6 [PMID: 20510161 DOI: 10.1016/j.bbrc.2010.05.082]

135 **Epis MR**, Giles KM, Kalinowski FC, Barker A, Cohen RJ, Leedman PJ. Regulation of expression of deoxyhypusine hydroxylase (DOHH), the enzyme that catalyzes the activation of eIF5A, by miR-331-3p and miR-642-5p in prostate cancer cells. *J Biol Chem* 2012; **287**: 35251-35259 [PMID: 22908221 DOI: 10.1074/jbc.M112.374686]

136 **Thorgeirsson SS**, Lee JS, Grisham JW. Functional genomics of hepatocellular carcinoma. *Hepatology* 2006; **43**: S145-S150 [PMID: 16447291 DOI: 10.1002/hep.21063]

137 **Galuppo R**, Ramaiah D, Ponte OM, Gedaly R. Molecular therapies in hepatocellular carcinoma: what can we target? *Dig Dis Sci* 2014; **59**: 1688-1697 [PMID: 24573715 DOI: 10.1007/s10620-014-3058-x]

138 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/s1470-2045(08)70285-7]

139 **Zhao YJ**, Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. *Mol Clin Oncol* 2013; **1**: 593-598 [PMID: 24649215 DOI: 10.3892/mco.2013.119]

140 **Kalari S**, Pfeifer GP. Identification of driver and passenger DNA methylation in cancer by epigenomic analysis. *Adv Genet* 2010; **70**: 277-308 [PMID: 20920752 DOI: 10.1016/b978-0-12-380866-0.60010-1]

141 **Kaminskas E**, Farrell A, Abraham S, Baird A, Hsieh LS, Lee SL, Leighton JK, Patel H, Rahman A, Sridhara R, Wang YC, Pazdur R. Approval summary: azacitidine for treatment of myelodysplastic syndrome subtypes. *Clin Cancer Res* 2005; **11**: 3604-3608 [PMID: 15897554 DOI: 10.1158/1078-0432.ccr-04-2135]

142 **Hellebrekers DM**, Griffioen AW, van Engeland M. Dual targeting of epigenetic therapy in cancer. *Biochim Biophys Acta* 2007; **1775**: 76-91 [PMID: 16930846 DOI: 10.1016/j.bbcan.2006.07.003]

**P-Reviewer:** Lampiasi N, Ng IOL, Varona MA **S-Editor:** Yu J

**L-Editor:** **E-Editor:**

**Table 1 Genes upregulated in hepatocellular carcinoma; putative oncogenes**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Symbol****(location)** | **Biological function** | **Expression** | ***n*** | **Relevant clinical factors** | **Functional analyses** | **Interacting molecules** | ***In vivo*** | **Ref.** |
| *AURKA* (20q13) | Cell cycle-regulated kinase | IHC | 44 | OS, RFS, number, stage | Chemoresistance, apoptosis | NF-κB, miRNA-21, PTEN | Yes | 40 |
| *BIRC7* (20q13.3) | Inhibitor of apoptosis protein family | mRNA, IHC, WB | 61 | None | Proliferation, migration, invasion, apoptosis | CDK | - | 21 |
| *Brachyury* (6q27) | Embryonic nuclear transcription factor | mRNA, IHC | 112 | OS, size, stage | Migration, invasion | PTEN, Akt, Snail, EMT | - | 22 |
| *CCL20* (2q36.3) | Cytokine displaying chemotactic activity for lymphocytes | IHC | 62 | OS, RFS, AFP, size, mumber, vascular invasion, differentiation | Proliferation, migration | pAKT, β-catenin, EMT | - | 45 |
| *CCT8* (21q22.11) | Transport and assembly of newly synthesized proteins | IHC | 102 | OS, grade, size | Proliferation, cell cycle | - | - | 30 |
| *CTSL* (9q21.33) | A lysosomal cysteine proteinase for intracellular protein catabolism | mRNA, IHC, WB | 82 | OS, RFS, stage, differentiation | Proliferation, tumorigenesis | - | Yes | 36 |
| *CYTH3* (7p22.1) | Mediator of protein sorting and membrane trafficking | mRNA, IHC | 202 | OS, size, vascular invasion | Proliferation, migration | - | - | 24 |
| *DLX4* (17q21.33) | Forebrain and craniofacial development | mRNA, IHC, WB | 226 | OS, size, differentiation, AFP | - | - | - | 27 |
| *GOLPH3* (5p13.3) | Peripheral membrane protein of the Golgi stack regulating Golgi trafficking | mRNA, IHC | 173 | OS, stage | Chemoresistance, apoptosis, angiogenesis, proliferation | NF-κB | Yes | 44 |
| *GSC* (14q32.1) | Autoregulatory transcription factor | mRNA, IHC, WB | 112 | OS, distant metastasis | Migration, invasion | EMT | - | 39 |
| *HSF1* (8q24.3) | Heat-shock transcription factor rapidly induced after temperature stress | IHC | 67 | OS | Proliferation | - | - | 34 |
| *IL-6*(7p21) | Cytokine that functions in inflammation and the maturation of B cells | mRNA, IHC, WB, ELISA | 120 | RFS, vascular invasion, number | Stemness | IGFIR, OCT4, NANOG | Yes | 43 |
| *ISG15* (1p36.33) | Chemotactic activity towards neutrophils | mRNA, IHC | 50 | OS, differentiation, distant metastasis | Proliferation, migration | Survivin | Yes | 33 |
| *KPNA2* (17q24.2) | Nuclear transporter of proteins and VJ recombination | mRNA, IHC | 314 | OS, RFS, size | Proliferation, metastasis | PLAG1 | - | 29 |
| *LGALS3* (14q22.3) | Member of the galectin family of carbohydrate binding proteins | mRNA, IHC, WB | 165 | OS, microvessel density | Proliferation, migration, invasion, apoptosis | - | - | 31 |
| *MACC1* (7p21.1) | Regulator of *HGF*-*HGFR* pathway | mRNA, IHC, WB | 50 | OS, grade, stage | Proliferation, apoptosis | HGF, c-MET, PI3K, AKT, Caspase 9 | Yes | 47 |
| *MAGED2* (Xp11.21) | Promotor of the cancer cell adhesion to the vascular epithelium | mRNA, IHC | 151 | OS | - | - | - | 51 |
| *MAGED4* (Xp11.22) | Unknown | mRNA, IHC | 94 | OS, RFS, AFP, vascular invasion, differentiation | - | - | - | 50 |
| *MCT4* (17q25) | Catalyzing lactic acid and pyruvate transport across plasma membranes. | IHC | 318 | OS, RFS, AFP, size | Proliferation, migration, invasion | pAKT, HIF-1α | - | 26 |
| *MRC2* (17q23.2) | Extracellular matrix remodeling by mediating the internalization | IHC | 96 | OS, number, vascular invasion | Migration, invasion | TGFb | - | 25 |
| *PDGFRα* (4q12) | Cell surface tyrosine kinase receptor for platelet-derived growth factor family | mRNA | 57 | OS, RFS, vascular invasion, microvessel density | Proliferation, migration, invasion | EMT | Yes | 38 |
| *PKM2* (15q22) | Pyruvate kinase generating ATP and pyruvate | mRNA, IHC, WB | 721 | OS, RFS, size, vascular invasion, differentiation | Proliferation, migration, invasion | pAKT, HIF-1α | Yes | 46 |
| *PLK1* (16p12.2) | Controlling mitotic entry, centrosome maturation, and bipolar spindle formation | IHC | 67 | Differentiation, capsule invasion | Proliferation, cell cycle, apoptosis | caspase3/8, Bax, Bcl-2 | - | 37 |
| *PTP4A1* (6q12) | Regulator of cellular processes, including cell proliferation and migration | IHC | 167 | OS | Migration, invasion | PI3K/AKT/GSK3β pathway | - | 32 |
| *SERPINB3* (18q21.3) | Ovalbumin family of serine proteinase inhibitors | mRNA, IHC | 67 | RFS | - | HIF-2α | - | 41 |
| *SIRT1* (10q21.3) | Regulating epigenetic gene silencing and suppress recombination of rDNA | mRNA, IHC, WB | 99 | OS, size, number, stage | Migration, invasion | EMT | Yes | 28 |
| mRNA | - | - | Proliferation, cellcycle | miR29c | - | 58 |
| IHC | 248 | RFS | Proliferation, cell cycle | p53, AMPK, mTOR | - | 59 |
| *TPX2* (20q11.2) | Microtubule organization during mitosis | mRNA, IHC | 130 | OS, RFS, stage, number, differentiation | Migration, invasion | MMP2/9, pAKT | - | 35 |
| mRNA, IHC | 86 | OS, RFS, vascular invasion, stage | Proliferation |  | Yes | 62 |
| *TREM1* (6p21.1) | Receptor belonging to the Ig superfamily that is expressed on myeloid cells | mRNA, IHC, WB | 322 | OS, RFS, age, AFP | Proliferation, invasion, cell cycle, apoptosis | IL-1β, TNF-α, MCP-1, p65, STAT3, ERK | - | 23 |
| *UBE2Q1* (1q21.3) | Member of the E2 ubiquitin-conjugating enzyme family | IHC | 86 | OS, grade | Proliferation, cell cycle | p53, p21 | - | 42 |
| *UBE4B* (1p36.3) | Conjugation factor E4 involved in multiubiquitin chain assembly | mRNA, IHC, WB | 149 | OS, grade, stage | Proliferation, migration, invasion, apoptosis | p53, Bcl-2, caspase 3 | - | 48 |
| *VEGF-B* (11q13) | Regulator of angiogenesis | mRNA | 48 | Number, vascular invasion, capsule invasion, stage | - | - | - | 12 |
| *YY1AP1* (1q22) | Unknown | Microarray | 76 | OS | Proliferation, apoptosis, spheroid-formation | EpCAM | Yes | 49 |

IHC: Immunohistochemistry; WB: Western blotting; OS: Overall survival; RFS: Recurrence free survival; AFP: Alpha-fetoprotein; PIVKA: Protein induced by vitamin K antagonists.

**Table 2 Genes suppressed in hepatocellular carcinoma; putative tumor suppressors**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Symbol****(location)** | **Biological function** | **Expression** | ***n*** | **Relevant clinical** **factors** | **Functional analyses** | **Interacting molecules** | ***In vivo*** | **Ref..** |
| *ARNT2*(15q24) | Partner for several sensor proteins of the bHLH-PAS family | IHC | 195 | OS, RFS, age, size, number, vascular invasion, AFP, differentiation, stage | Proliferatio, invasion, migration | - | Yes | 79 |
| *BTG1* (12q22) | Regulates cell growth and differentiation | mRNA, IHC | 151 | OS, RFS, PIVKA-II, size, differentiation, vascular invasion, stage, extra‑hepatic recurrence | - | - | - | 80 |
| *CDH3* (16q22.1) | Calcium-dependent cell-cell adhesion glycoprotein | IHC | 69 | Stage | Proliferation | - | - | 71 |
| *EBP1* (12q13.2) | RNA-binding protein involved in cell growth regulation | IHC, WB | 103 | OS, grade, size | Growth, cell cycle | - | - | 74 |
| *FBLN5* (14q32.1) | Secreted extracellular matrix protein | mRNA, IHC | 86 | OS, number, vascular invasion, grade, stage | Migration, invasion | MMP7 | - | 76 |
| *Fbxw7* (4q31.3) | Ubiquitin-mediated degradation of cyclin E | IHC | 60 | OS, size, vascular invasion, grade, stage | Growth, apoptosis | YAP | Yes | 77 |
| *GPx3*(5q23) | Detoxification of hydrogen peroxide | mRNA, IHC, ELISA | 113 | OS, RFS, size, number, vascular invasion, stage | Proliferation, invasion | Erk, NFκB, SIP1 | Yes | 75 |
| *IGFBP-1* (7p12.3) | Mediator of the IGFs | IHC | 90 | OS, differentiation, cirrhosis, vascular invasion, stage | Invasion | MMP9 | - | 73 |
| *LGALS4* (19q13.2) | Modulating cell-cell and cell-matrix interactions | mRNA, IHC, ELISA | 201 | Size, differentiation, vascular invasion, stage | Migration, invasion | - | - | 72 |
| *TIPE1* (19p13.3) | Unknown | IHC | 50 | OS, grade | Growth, apoptosis | Nec-1, Rac-1 | Yes | 78 |

IHC: Immunohistochemistry; WB: Western blotting; OS: Overall survival; RFS: Recurrence free survival; AFP: Alpha-fetoprotein; PIVKA: Protein induced by vitamin K antagonists.

**Table 3 Genes epigenetically suppressed in hepatocellular carcinoma through promoter hypermethylation**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Symbol****(location)** | **Biological function** | **Expression** | ***n*** | **Relevant clinical factors** | **Functional analyses** | **Interacting molecules** | ***In vivo*** | **Ref.** |
| *CLDN3* (7q11.23) | Integral membrane protein and a component of tight junction strands | mRNA, IHC | 114 | OS | Motility, invasion, tumor formation | Wnt, EMT, β-catenin | Yes | 99 |
| *DENND2D*(1p13.3) | Membrane trafficking protein regulating Rab GTPases | mRNA, IHC | 92 | OS, RFS | - | - | - | 100 |
| *DPT* (1q12-q23) | Cell-matrix interactions and matrix assembly | mRNA, IHC, WB | 202 | OS, RFS, AFP, vascular invasion, differentiation | Migration, metastasis | RhoA, FAK, c-SRC, α3β1 integrin | Yes | 98 |
| *DPYSL3* (5q32) | Cell-adhesion factor involved in the metastatic process of tumor cells | mRNA, IHC | 151 | OS, RFS, AFP, PIVKAII, gender, vascular invasion, serosal invasion | Migration, invasion | VEGF, FAK | - | 105 |
| *EFEMP1* (2p16) | Extracellular matrix glycoproteins | mRNA | 48 | OS, liver damage, AFP | - | - | - | 103 |
| *FBLN1* (22q13) | Secreted fibrillar extracellular matrix protein | mRNA, IHC, WB | 48 | number, size, stage | - | - | - | 96 |
| *MEG3* (14q32) | Unknown | mRNA | 72 | OS, RFS, tumor size and Edmondson grade | Proliferation, apoptosis | p53, UHRF1 | Yes | 102 |
| *MT1G* (16q13) | Preserve the homeostasis of metals | mRNA | 48 | none | - | - | - | 11 |
| *PDSS2* (6q16.3-21) | Synthesis of coenzyme Q10 | mRNA, IHC | 151 | OS, RFS, AFP, vascular invasion, differentiation, serosal invasion, stage | - | HNF4a, CDX2 | - | 101 |
| *RELN*(7q22) | Secreted extracellular matrix protein | mRNA | 48 | RFS | - | - | - | 104 |
| *TIF1γ* (7p22.1) | Unknown | IHC, WB | 204 | OS, RFS, stage | Invasion, metastasis | TGF-β/Smad | - | 97 |
| *TUSC1* (9p21.2) | Unknown | mRNA, IHC | 94 | OS, stage | - | - | - | 106 |

IHC: Immunohistochemistry; WB: Western blotting; OS: Overall survival; RFS: Recurrence free survival; AFP: Alpha-fetoprotein; PIVKA: Protein induced by vitamin K antagonists.

**Table 4 Dysregulated microRNAs in hepatocellular carcinoma**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **microRNA** | **Sample** | ***n*** | **Relevant clinical factors** | **Functional analyses** | **Interacting molecules** | ***In vivo*** | **Ref.** |
| miR-9 | Tissue | 200 | OS, vascular invasion, stage | - | - | - | 122 |
| miR-21 | Tissue | 109 | RFS, cirrhosis, stage | Growth | AP-1 | Yes | 129 |
| miR-106b | Tissue | 104 | OS, size, vascular invasion | - | - | - | 125 |
| miR-122, miR-29 | Serum | 20 | Liver damage | - | - | - | 127 |
| miR-128-2 | Tissue, serum | 182 | OS, liver damage | - | - | - | 132 |
| miR-130a | Tissue | 102 | OS, gender, HBsAg status, size, stage | - | - | - | 124 |
| miR-130b | Tissue | 97 | OS, RFS, HBsAg status, AFP, size, grade, stage | - | - | - | 126 |
| miR-138 | Tissue | 180 | OS, stage, vascular invasion, stage | - | cyclin D3 | - | 130 |
| miR-148a | Tissue | 297 | OS, size | Proliferation, migration, invasion | ACVR1, BMP, Wnt | Yes | 131 |
| miR-224 | Tissue | 130 | OS, RFS, AFP, stage, grade | - | pAKT | - | 128 |
| miR-331-3p | Tissue | 108 | OS, RFS, size, number, vascular invasion | Proliferation, migation, metastasis | PHLPP, AKT, EMT | Yes | 123 |

miR: MicroRNA; OS: Overall survival; RFS: Recurrence free survival; HBsAg: Hepatitis B surface antigen; AFP: Alpha-fetoprotein.