

Review

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Oncogenic Wnt3a: a promising specific biomarker in hepatocellular carcinoma

Min Yao^{1,2,#}, Miao Fang^{1,#}, Wen-Jie Zheng¹, Deng-Fu Yao¹

¹Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China.

²Department of Immunology, Medical School of Nantong University, Nantong 226001, Jiangsu, China.

#Authors contributed equally.

Correspondence to: Prof. Deng-Fu Yao, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China. E-mail: yaodf@ahnmc.com

How to cite this article: Yao M, Fang M, Zheng WJ, Yao DF. Oncogenic Wnt3a: a promising specific biomarker in hepatocellular carcinoma. *Hepatoma Res* 2018;4:30. <http://dx.doi.org/10.20517/2394-5079.2018.32>

Received: 28 Mar 2018 **First Decision:** 22 May 2018 **Revised:** 11 Jun 2018 **Accepted:** 11 Jun 2018 **Published:** 6 Jul 2018

Science Editor: Guang-Wen Cao **Copy Editor:** Jun-Yao Li **Production Editor:** Cai-Hong Wang

Abstract

Hepatocellular carcinoma (HCC) is still one of the most common and rapidly fatal malignancies worldwide with a multi-factorial, multi-step, complex process, and poor prognosis. Early discovery and effective therapy of HCC are of utmost importance. Recent studies demonstrated that Wnt/ β -catenin pathway play important roles in occurrence and development of HCC including hepatocytes malignant transformation, metastasis, chemoresistance and liver cancer stem cells. Oncogenic wingless-type MMTV integration site family member 3a (Wnt3a) signaling is a promising biomarker in diagnosis and prognosis for HCC. This review presents current data on mechanisms of hepatocarcinogenesis involving participation of the Wnt canonical pathway, and focuses on the Wnt3a expression in HCC progression and its clinical application.

Keywords: Hepatocellular carcinoma, Wnt/ β -catenin pathway, signal molecules

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common or deathly human malignancy cancers worldwide^[1,2], especially in the areas along the Yangtze River. Recently, Chen *et al.*^[3] reported the observed survival and relative survival of leading cancer sites from a population-based cancer registry for 40 years. The main sites of the cancer types with a total of 92,780 incident cases in Qidong, China, HCC ranks the first based on the rank order of incidence among all malignancies (liver, stomach, lung, colon and rectum, oesophagus, breast, pancreas, leukaemia, brain and central nervous system, bladder, non-Hodgkin's



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Table 1. Chromosomal location of Wnt genes and tissue distribution

Gene	Location	Accession numbers	Tissues or tumors
<i>Wnt 1</i> ^[22]	12q13	X03072	Lipomas, myxoid liposarcomas, pleomorphic adenomas, myomas
<i>Wnt 2</i> ^[23]	7q31	X07876	Lung, heart
<i>Wnt 2b/13</i> ^[24]	1p13	XM052111, XM052112	Cervical cancer, gastric cancer
<i>Wnt 3</i> ^[25]	17q21	AY009397	Breast
<i>Wnt 3a</i> ^[26]	1q42.13	AB060284	Spinal cord, brain, liver
<i>Wnt 4</i> ^[27]	1p35	AY009398	Breast
<i>Wnt 5a</i> ^[28]	3p14-p21	L20861	Neonatal heart, lung, liver
<i>Wnt 5b</i> ^[29]	12p13.3	AB060966	Prostate, fetal brain & lung, kidney, liver, ovary, small intestine
<i>Wnt 6</i> ^[30]	2q35	AY009401	Kidney, placenta, spleen
<i>Wnt 7a</i> ^[31]	3p25	D83175	Placenta, kidney, testis, uterus, fetal lung, brain
<i>Wnt 7b</i> ^[32]	22q13.3	AB062766	Brain, kidney, prostate, lung, esophageal, gastric, pancreatic cancer
<i>Wnt 8a/d</i> ^[33]	5q31	AB057725, AY009402	Teratocarcinoma, mesoderm
<i>Wnt 8b</i> ^[34]	10q24	Y11094	Forebrain
<i>Wnt 10a</i> ^[30]	2q35	AB059569	Kidney, placenta, spleen, brain, liver
<i>Wnt 10b/12</i> ^[35]	12q13.1	U81787	Lung, uterus, thymus, spleen, breast
<i>Wnt 11</i> ^[36]	11q13.5	Y12692	Skeleton, lung
<i>Wnt 14</i> ^[37]	1q42	AB060283	Breast
<i>Wnt 15</i> ^[37]	17q21	AF028703	Breast
<i>Wnt 16</i> ^[38]	7q31	XM031374, XM00488	Spleen, appendix, lymph nodes

lymphoma, and cervix) and the poorest survival rate^[3]. The leading etiological factors of HCC include chronic hepatitis B or C virus (HBV^[4-6] or HCV^[7,8]) infection, aflatoxin contaminated food taken and non-alcohol fat liver diseases (NAFLD)^[9,10]. Chronic HBV carriers have a 5-15-fold increased risk of HCC compared with the general population. HBV-related proteins are known to take control of several cellular pathways like Wnt/ β -catenin, TGF- β , Raf/MAPK, and ROS for the virus's own replication^[11-13].

Carcinogenesis of HCC is a multi-factor, multi-step and complex process. Most of HCC patients died quickly because of the rapid tumor progression, and hepatic resection or transplantation is the only potential curative treatment for HCC patients^[14,15]. Activation of the Wnt/ β -catenin signaling pathway plays a significant role in the pathology and physiology of the liver and has been identified as a main factor in HCC because of hepatocytes malignant transformation with numerous genetic/epigenetic abnormalities, and affects cellular persistence, multiplication, migration, alteration and genomic instability^[16-18]. Abnormal expressions of Wnt signaling molecules were closely associated with the occurrence and progression of HCC. Recently, Pan *et al.*^[19,20] discovered and reported that the overexpression of oncogenic wingless-type MMTV integration site family member 3a (Wnt3a) could be a specific biomarker in diagnosis and prognosis of HCC. However, its exact underlying mechanisms in hepatocarcinogenesis still remain poorly understood. This review presents new advances of the underlying mechanisms of Wnt signaling, and focuses on expressions of hepatic or circulating Wnt3a, which serve as a promising molecular biomarker for HCC.

REGULATING MECHANISMS OF Wnt SIGNALINGS

Human Wnt genes encode a large family of secreted proteins that have been reported in many tissues^[21]. Total 19 Wnt proteins in human tissues or cancers are shown in Table 1. Proteins were identified that share 27% to 83% amino acid sequence identity, and evolutionarily conserved glycoproteins with 23 or 24 cysteine residues. Human Wnt proteins are all very similar in size, ranging in molecular weight from 39 kDa (Wnt7a) to 46 kDa (Wnt10a). Wnt protein folding may depend on the formation of multiple intramolecular disulfide

bonds. Analysis of the signaling activities of chimeric Wnt proteins has shown that the carboxy-terminal region of Wnt proteins may play a role in determining the specificity of responses to different Wnts. The amino-terminal region may mediate interactions with Wnt receptors but requires the carboxyl terminus to activate these receptors. The main regulating mechanisms of Wnt signaling are either through canonical pathway (Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a, and Wnt10b) characterized by the stabilization and subsequent nuclear transport of β -catenin resulting in the activation of transcriptional responses or via non-canonical pathway (Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b, and Wnt11) with more diverse and several different signaling modes that regulate cell biological behaviors^[22-38].

The Wnt signaling molecules have been involved in liver tumorigenesis with activating liver cancer stem cells^[39]. In adults, Wnts function in homeostasis, and inappropriate activation of the Wnt pathway is implicated in a variety of cancers. Some signaling molecules in the Wnt pathway have been recognized to play an important role in the development and progression of tumors and regulate multiple cellular events such as cell proliferation, differentiation, and apoptosis through β -catenin-dependent canonical- or β -catenin-independent noncanonical pathway^[40]. Abnormal expression of some key molecules in the Wnt/ β -catenin pathway was associated with the development and progression of HCC. Wnt3a gene located on chromosome (1q42.13) has been regarded as an activator inducing β -catenin accumulation and activating the canonical Wnt signaling pathway. Studies on human Wnt3a have focused primarily on its key role in liver malignancy, and its high expression in cancerous tissues has been confirmed with a worse outcome^[20].

HBV INVOLVED IN Wnt ACTIVATION

HBV has a global distribution and is one of the leading causes of HCC. Its viral replication with several pathways like Wnt/ β -catenin, TGF- β , Raf/MAPK and ROS affects cellular persistence, multiplication, migration, alteration and genomic instability^[41,42]. The Wnt/FZD/ β -catenin pathway associated with HBV-related HCC development because of the progression of chronic liver diseases is known to be accompanied by disturbances in β -catenin expression (mainly overexpression)^[43,44], with its cytoplasmic or nuclear translocation. Viral proteins of HBV (HBx and HBsAg) can act as pathogenic factors that are involved in the modulation and induction of canonical Wnt signaling activation with aberration of adenomatous polyposis coli (APC), AXIN, secreted Frizzled related protein (SFRP) 1 and SFRP5.

The canonical Wnt signals are transduced through Frizzled receptors and LRP5/LRP6 co-receptors located on the cell membrane, initiating the β -catenin signaling cascade^[45,46]. This multi-protein destruction complex could target the proto-oncogene β -catenin for ubiquitin-mediated proteolysis, prevent glycogen synthase kinase 3 α (GSK-3 α)-mediated β -catenin degradation, leading to nuclear translocation of β -catenin, combine with T-cell factor/lymphoid enhancer factor, and thereby promote the transcription of downstream target genes, including *FGF20*, *DKK1*, *WISP1*, *MYC*, *CCND1*, and so on. Their interaction results in the enhancement of the pathway and leads to hepatocarcinogenesis^[47,48]. Thus, lack of Wnt secretion from hepatocytes did not affect overall injury, fibrosis or HCC burden although there were protein expression differences in tumor conformation^[49].

HCV PROVOKED Wnt SIGNALING

Epidemiological studies have validated the association between HCV infection and HCC. An increasing number of studies show that protein-protein interactions between HCV proteins and host proteins play a vital role in infection and mediate HCC progression^[50]. The role of nonstructural (NS5A) protein of HCV *in vivo* has been accentuated in induction of this pathway mainly to the canonical pathway. Interaction of Wnt signaling with HCV genome in hepatocarcinogenesis linked β -catenin phosphorylation and abnormalities in the E-cadherin-catenin unit function lead to loss of intercellular junctions, progression in liver fibrosis, and development of cirrhosis and HCC^[51,52]. Accumulating evidence indicates that HCV core or nonstructural

proteins provoke activation of the Wnt/ β -catenin signaling pathway, and the evidence supporting a role of Wnt/ β -catenin signaling in the onset and progression of HCC is compelling^[53,54].

Progression of HCV-related liver diseases is noted to be accompanied by disturbances in β -catenin overexpression, with its cytoplasmic or nuclear translocation and with lower expression of E-cadherin. More β -catenin mutations are manifested in HCV-associated than in HBV-related HCC. HCV proteins affect in a double manner expression of E-cadherin, including modulation of the Wnt pathway and reduction of E-cadherin expression at the transcriptional level. Alterations in cellular locations of β -catenin and E-cadherin in chronic HCV and HCC pointed to structural disturbances in intercellular junctions in livers and presence of the transcriptionally inactive form of β -catenin^[55,56]. Promoter hypermethylation of Wnt inhibitors was discovered in HCV-induced multistep hepatocarcinogenesis^[57], and the reduced expression of E-cadherin in long-lasting chronic HCV might represent an early indicator of the epithelial-mesenchymal transition^[58,59].

COUNTERACTIVE Wnt3a WITH Wnt5a IN HCC

Although accumulating clinical and basic evidences have suggested that the Wnt signaling is associated with the HCC progression^[60]. However, little research has been reported on the relationship between Wnt3a and HCC. Previous studies have found that Wnt3a showed higher expression in HCC than liver tissues, positively correlated with its target genes MMP 7 and c Myc. Intriguingly, their expressions are significantly correlated with Notch3 and Hes1 expression. Wnt3a was highly expressed in MHcc97H and SK Hep 1 cells *in vitro*^[61], as an important regulator of human HCC cell line growth, which could induce activation of the canonical Wnt pathway after binding with SULF2 and GPC-3. Also, it could increase cell proliferation in nude mouse xenografts *in vivo*^[60,61].

The expressions of hepatic Wnt3a were investigated in HCC tissues [Figure 1]. The positive Wnt3a with brown staining particles was mainly distributed in cytosol and membrane of hepatocytes in cancerous tissues and no or lower expression in their surrounding tissues. High Wnt3a expression like its down-stream disheveled 2, DKK1, and SFRP1 were all identified as independent predictive factors for poor HCC outcome^[20,62-64]. Compared with high hepatic Wnt3a in HCC tissues, the significant difference of Wnt5a intensity was found between low level in HCC tissues and high expression in their para-cancerous tissues. The intensity of Wnt5a expression was inversely correlated with Wnt3a level in cancerous tissues. Both decreasing Wnt5a and increasing Wnt3a expression in HCC tissues relation to the clinical staging from stage I to IV were confirmed as independent prognosis factors of HCC patients. The Kaplan-Meier survival curves demonstrated that HCC patients with high Wnt3a expression had a significantly lower survival rate compared to cases with lower Wnt3a [Figure 2], Wnt3a expression was associated with poorly-differentiated grade, liver cirrhosis, chronic HBV infection, and higher TNM stage, indicating that the abnormal Wnt3a expression could participate in promoting hepatocytes malignant transformation and progression of HCC^[65,66].

SERUM Wnt3a FOR HCC SPECIFIC DIAGNOSIS

Early diagnosis of HCC is of the utmost importance. Successful screening for HCC at early stage is challenging due to the lack of well characterized and specific biomarkers^[67,68]. Data of previous studies have confirmed that some Wnt signalings could modify HCC growth and invasive ability. However, achieving successful screening of abnormal Wnt3a signaling is critically important as early diagnosis could potentially provide an early monitoring opportunity. Along these lines, the Wnt pathway has been identified as contributing to the development and progression of HCC. Although serological AFP marker is commonly applied to HCC diagnosis, it has exhibited a low sensitivity and specificity with approximately 40% of negative patients. Although many biomarkers have been applied in diagnosis for HCC, only a few markers were confirmed with higher specificity or sensitivity for HCC, especially in early stage or small size HCC^[69-71].

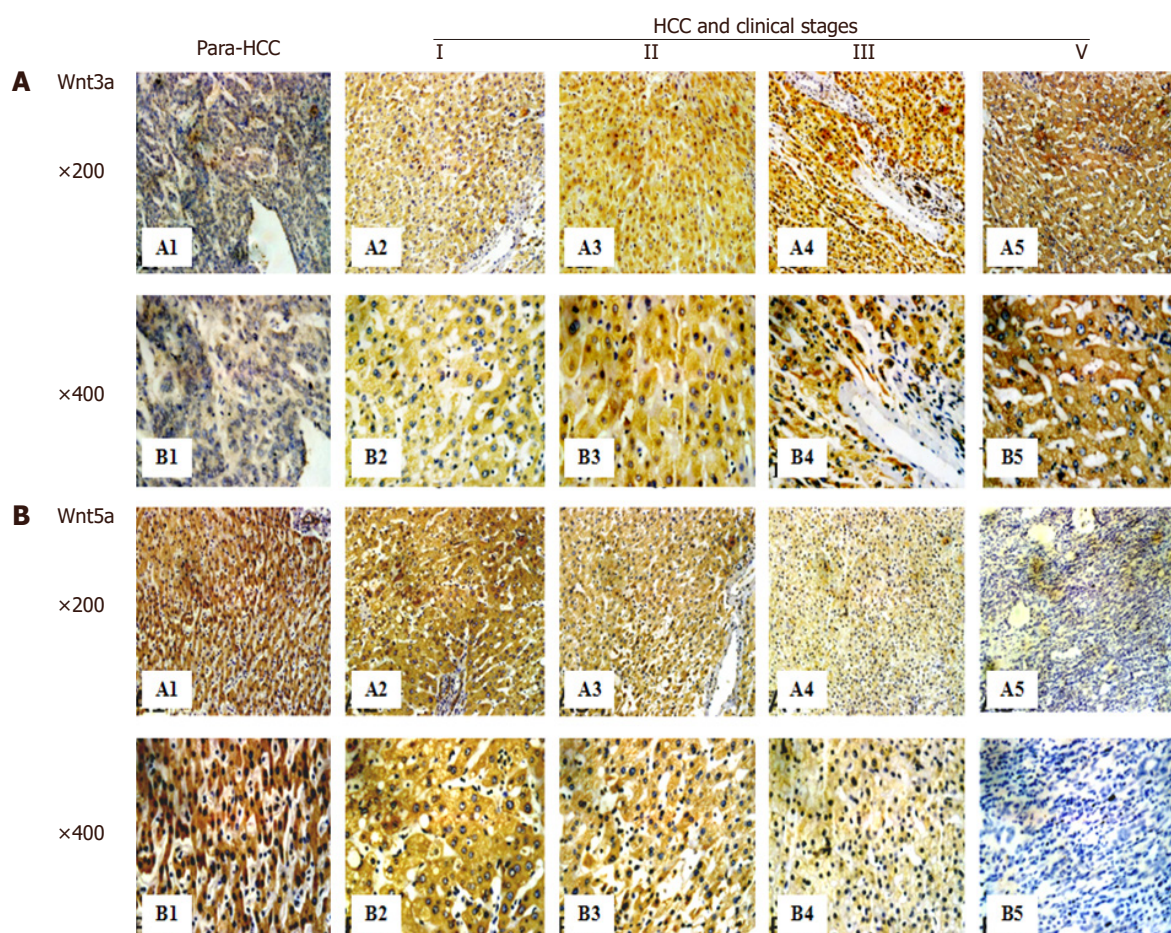


Figure 1. Immunohistochemistry of Wnt3a or Wnt5a expression in HCC tissues with different staging^[20,65]. (A) The Wnt3a expression in HCC tissues (SP, original magnification: A1-A5, $\times 200$; B1-B5, $\times 400$); (B) the Wnt5a expression in HCC tissue (SP, original magnification, A1-A5, $\times 200$; B1-B5, $\times 400$). In Wnt3a, A1 and B1, the low or without Wnt3a expression in the para-cancerous tissues, and A2-A5 and B2-B5, the brown staining of Wnt3a expression with gradually increasing from stage I, II to III-IV of HCC tissues; In Wnt5a, A1 and B1, the strongest Wnt3a expression in the para-cancerous tissues, and A2-A4 and B2-B4, the brown staining of Wnt3a expression with gradually decreasing from stage I to III of HCC tissues, and A5 and B5, the low or without Wnt5a expression were discovered in HCC tissues at stage IV. HCC: hepatocellular carcinoma tissues; Para-HCC: paracancerous tissues; Wnt3a: wingless-type MMTV integration site family member 3a; Wnt5a: wingless-type MMTV integration site family member 5a

Cancerous Wnt3a was over-expressed and could secrete into circulating blood. The incidence of serum Wnt3a level (> 800 ng/L) in HCC patients was 92.5% with significantly related to AFP level, liver cirrhosis, HBV infection, low differentiation degree, TNM staging, and extra-hepatic metastasis^[19]. According to the diagnostic specificity or the area under the receiver operating characteristic (ROC) curve, serological Wnt3a detection has been confirmed superior to AFP, HS-GGT^[72], and GPC-3^[73] with higher sensitivity and lower false-positive rate for HBV-related HCC patients [Table 2]. The combining of serum Wnt3a plus AFP detection has complemented diagnostic value and raised the sensitivity up to 96.3% for HCC diagnosis which was obviously higher in Wnt3a or AFP alone for distinguishing malignancy from benign liver lesions, suggesting that serum Wnt3a should be a novel specific marker for HCC diagnosis that was superior to routine AFP detection^[74] according to the specificity and the area under the ROC curve, especially in diagnosis of AFP-negative HCC.

Wnt3a SIGNALING WITH HCC TARGETED-THERAPY

Once HCC is advanced, there are multiple therapeutic venues, but most eventually fail. Effective treatment of HCC still is a challenging problem worldwide. Therefore, developing novel molecule-targeted therapies may

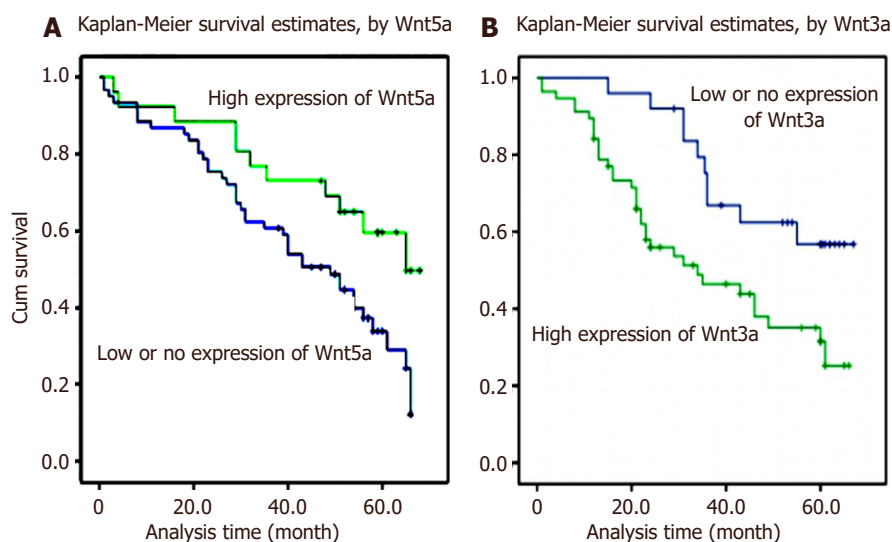


Figure 2. Overall survival curves of Wnt5a or Wnt3a expression in HCC^[20,65]. The hepatic Wnt5a or Wnt3a expression curves were calculated according to the Kaplan-Meier method. The accumulative survival curves of patients with HCC were made according to HCC tissues with low or high expression for Wnt5a or Wnt3a level (log-rank test, $P < 0.001$). (A) Wnt5a in HCC; (B) Wnt3a in HCC. HCC: hepatocellular carcinoma; Wnt3a: wingless-type MMTV integration site family member 3a; Wnt5a: wingless-type MMTV integration site family member 5a

Table 2. Comparative analysis of circulating Wnt3a, AFP, HS-GGT, and GPC-3 detection in diagnosis of HCC

	Wnt3a ^[19] (> 800 ng/L)	AFP ^[19] (> 50 ng/mL)	HS-GGT ^[17] (> 5.5 U/L)	GPC-3 ^[67] (positive)	Wnt3a ^[19] + AFP
Sensitivity (%)	92.50	61.25	85.70	52.84	96.25
Specificity (%)	94.34	69.81	97.24	99.58	62.26
Accuracy (%)	93.23	64.66	96.20	83.57	82.71
PPV (%)	96.10	75.38	89.70	98.48	79.38
NPV (%)	89.29	54.41	92.23	80.20	91.67

Wnt3a + AFP: combining detection of serum Wnt3a and AFP concentration; Wnt3a ($n = 80$), AFP ($n = 80$), HS-GGT ($n = 91$), and GPC-3 ($n = 123$). PPV: positive predictive value; NPV: negative predictive value; HCC: hepatocellular carcinoma; GPC-3: glypican-3; HS-GGT: HCC-specific gamma-glutamyl transferase; AFP: alpha fetoprotein

provide greater chance for effective therapies^[75] or overcoming resistance to sorafenib^[76]. Many mechanisms have been involved in the aberrant activation of Wnt signaling and regulating β -catenin activity^[77] or function by using small molecules (LGK974^[78], Celecoxib^[79], Genistein^[80]), specific antibodies (OMP-54F28, OTSA101)^[81] and small size peptide SAH-BCL-9^[82]. However, only a few of anti-cancer drugs that have been developed to target the related pathway of HCC formation or development have entered into pre-clinical trials, and none of these have advanced to the late clinical trial stage.

Oncogenic Wnt3a is involved in HCC development and increasing Wnt3a plays a crucial role in cell proliferation and metastasis, particularly in progression and mediated-oncogenesis involving signaling pathways, with brown granule-like staining localized in cancerous parts of atypical hyperplasia^[19,20]. Targeted oncogenic glypican-3 gene transcription of Wnt upstream inhibited the proliferation of human hepatoma cells by specific short hairpin RNA^[83]. Down-regulating Wnt3a expression inhibited cell viability and induced G0/G1 cell cycle arrest via decreased expression of cyclin D1 and c Myc, and increased expression of p21 and p27. In addition, deletion of Wnt3a significantly inhibited migration and invasion by down-regulating MMP 2/-7/-9 expression via the MAPK (p38, ERK1/2 and JNK) pathway^[61]. The abnormality of liver and circulating Wnt3a expression in HCC has provided initial evidence, and suggested that targeted-Wnt3a signaling could be a promising target or an effective target for HCC therapy.

PERSPECTIVES

In conclusion, molecular factors are involved in the process of HCC development and metastasis. HBx could integrate into human genome and this transcript could activate Wnt signaling as a long noncoding RNA^[84]. The associations between Wnt signaling and cancer initiation, tumor growth, metastasis, dormancy, immunity and tumor stem cell maintenance have been revealed, and Wnt signaling has exhibited numerous genetic abnormalities^[85,86] as well as epigenetic alterations including modulation of DNA methylation. The overexpression of Wnt3a in cancerous tissues has been discovered, and its higher level was only found in sera of HCC patients from a cohort study in chronic liver diseases, although it is the first time to report as a novel specific marker for HCC diagnosis and prognosis. Further studies will permit us to analyze Wnt3a role in hepatocarcinogenesis and explore its molecular-targeted for HCC therapy^[87,88].

DECLARATIONS

Authors' contributions

Conception and literature search: Yao M, Zheng WJ

Drafting the manuscript: Yao M, Fang M

Critical revision for intellectual content: Yao DF

Availability of data and materials

Not applicable.

Financial support and sponsorship

This work was supported by the grants from the Projects of National Natural Science Foundation of China (81673241, 81702419), Jiangsu Medical Programs (KYCX17_1934, BE2016698), and Int. S&T Cooperation Program (2013DFA32150) of China.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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