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Review

Homeobox genes and hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer, and is the third leading cause of cancer-related deaths each year. It involves a multi-step progression and is strongly associated with chronic inflammation induced by the intake of environmental toxins and/or viral infections (i.e., hepatitis B and C viruses). Although several genetic dysregulations are considered to be involved in disease progression, the detailed regulatory mechanisms are not well defined. Homeobox (*Hox*) genes that encode transcription factors with homeodomains control cell growth, differentiation, and morphogenesis in embryonic development. Recently, more aberrant expressions of *Hox* genes were found in a wide variety of human cancer, including HCC. In this review, we summarize the currently available evidence related to the role of *Hox* genes in the development of HCC. The objective is to determine the roles of this conserved transcription factor family and its potential use as a therapeutic target in future investigations.

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Epidemiology

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancers and is ranked as the second leading cause of cancer-related mortality worldwide [1]. The global annual death of HCC was reported to be 700,000 patients [2]. Intriguingly, its incidence worldwide differs from heterogeneous prevalence of risk factors. The highest incidence of HCC has been reported in East/Southeast Asia and Africa and the lowest in South/Central Asia and Europe [3]. Commonly mentioned risk factors for HCC include chronic viral hepatitis (i.e., hepatitis B virus [HBV] and hepatitis C virus [HCV]), chronic alcohol use, environment pollutants, obesity, and diabetes mellitus. Chronic hepatitis B and exposure to aflatoxin are major risk factors for the occurrence of HCC in sub-Saharan Africa and Eastern Asia, whereas chronic hepatitis C is the major risk factor in the USA, Europe, and Japan [4]. In recent years, nonalcoholic fatty liver disease (NAFLD) has gradually emerged as a leading cause of HCC in Western and Asian populations [5].

Etiologies

Various possible mechanisms, which link these risk factors to hepatocarcinogenesis, have been proposed. HBV–an enveloped DNA virus–belongs to the *Hepadnaviridae* family and includes eight genotypes (i.e., A to H) which have their respective geographical distribution [6]. Studies have shown that the HBV X protein (HBx) is a 154-amino acid polypeptide that plays an essential role in the development of HCC. HBx regulates a diversity of cellular functions, including gene transcription, proliferation, protein degradation, and apoptosis. HBx may directly promote hepatocytes transforming into tumor-initiating cells through the activation of Wnt/β-catenin, which is a transcription coactivator [7]. HCV is a single-stranded positive RNA virus belonging to the *Hepacivirus* genus in the family Flaviviridae, including seven major genotypes [8]. HCV-induced progressive liver cirrhosis is a well-known risk factor for the development of HCC. Therefore, attaining sustained viral response through the use of appropriate therapy is essential. Moreover, it is important to note that a sustained viral response does not ensure the elimination of the risk of HCC, despite its substantially decreased incidence. Of note, HCC can occur more than 10 years after eradication of HCV, with an annual rate of 1% [9]. Liver cirrhosis is an established risk factor for HCC; it represents the final stage of liver fibrosis and usually develops in response to chronic liver injuries [10]. Chronic alcohol consumption and consequent liver cirrhosis play a causative role in the development of HCC. The activation of CYP2E1 induces hepatocarcinogenesis, which involves the generation of free radicals, producing DNA adducts and oxidative damage to the target cells [11].

Consumption of contaminated animal and plant products may expose individuals to aflatoxins, another common risk factor for the development of HCC. Aflatoxin B1 (AFB1) is the most potent liver carcinogen among the four aflatoxins (i.e., B1, B2, G1, and G2). *p53* gene mutations are associated with high exposure to AFB1. These mutations, such as codon 249 transversion, appeared in 50% of HCC cases. AFB1 is metabolized by cytochrome P450 to reactive genotoxic intermediate metabolites (i.e., aflatoxin B1-8, 9-oxide), which cause mutational effects by interacting with the guanine bases of DNA [12]. NAFLD encompasses a spectrum of pathological changes characterized by different degrees of fat accumulation in the hepatocytes. This condition is attributable to overnutrition and is strongly associated with metabolic syndrome. Nonalcoholic steatohepatitis (NASH) is a severe subtype of NAFLD with the histologic features of lobular inflammation and hepatocyte ballooning. Patients with NASH are predisposed to liver fibrosis, cirrhosis, and HCC [13]. Several mechanisms, including increased levels of tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and leptin, have been correlated with carcinogenesis from NASH.

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Treatments

In the past, HCC was usually diagnosed at an advanced stage, following the development of symptoms and impairment of liver function. At that point, treatment was often futile with poor median survival rates (i.e., <3 months) [14]. Currently, a substantial proportion of HCC patients continues to have a poor liver reserve and/or compromised portal vein flow. Thus, these untreated cases of HCC are associated with poor prognosis. With advancements in early HCC detection technology and surveillance programs, the curative treatment has improved the 5-year survival rates, ranging from 50% to 75%. [15]. Despite the availability of several therapeutic options for HCC (i.e., hepatic resection, liver transplantation, locoregional therapies, and systemic therapies), the treatment strategy must be individualized for each patient. The Barcelona Clinic Liver Cancer staging system is widely used worldwide to establish the prognosis and most appropriate treatment strategy for patients at different stages [16]. Although the so-called curative treatments (i.e., surgical resection, liver transplantation, and radiofrequency ablation) have greatly improved the outcomes of HCC, disease recurrence and intrahepatic metastasis continue to pose challenges in the treatment of these patients.

The Barcelona Clinic Liver Cancer algorithm suggests systemic treatment for advanced HCC. Sorafenib—an oral multikinase inhibitor of cell proliferation through a strong inhibition of the serine/threonine kinase RAF—is the first approved systemic medication for the treatment of advanced HCC. Moreover, it was shown to inhibit the pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor (PDGFR) [17,18]. The efficacy of sorafenib has been demonstrated in several clinical studies [19]. However, although sorafenib is currently considered the best option for treating advanced HCC, it only increases life expectancy by a few months. Recently, researchers focused on the development of other targeted therapies, such as the inhibition of angiogenesis, epidermal growth factor receptor, and mammalian target of rapamycin (mTOR) [20].

HCC develops several immunosuppressive mechanisms to evade the immunological surveillance system and progress further. The immune checkpoint regulation and its associated molecules advance to a new generation in cancer treatment. Among the different types of molecules involved in the immune checkpoints, programmed death-ligand 1 (PD-L1) found on the surface of cancer cells and stromal cells; programmed cell death 1 (PD-1); and cytotoxic T lymphocyte-associated protein 4 found on the surface of T cells have been shown to participate in the crucial steps for suppressed function of T-cell by cancer cells [21,22]. Active efforts toward immunotherapy for HCC include the development of monoclonal antibodies against molecules of the immune checkpoint. Numerous phase III trials are in progress. Among the investigated drugs, nivolumab is currently at the most advanced stage of clinical development, with phase III clinical data showing potential in the treatment of advanced HCC [23].

Homeobox (*Hox*) genes

Hox genes, which are master regulatory genes controlling the development of each segment, were firstly discovered in the fruit fly *Drosophila melanogaster* [24]. In flies, three *Hox* genes (*Ubx*, *Abd-A*, and *Abd-B*) belong to the bithorax complex, and other five *Hox* genes (*Lab*, *Pb*, *Dfd*, *Scr*, and *Antp*) belong to the antennapedia complex. *Hox* genes specify the regional identity from the anterior to the posterior of the body segments of the fly [25]. The concept that a cluster of regulatory genes may control the development of segments is conserved in a wide range of organisms (from *Caenorhabditis elegans* to humans). In humans, the *Hox* genes

are classified into four clusters (i.e., A to D) and located at 7p15, 17q21.2, 12q13, and 2q31. Based on sequence similarity and relative position of the cluster, each cluster is identified 13 paralog groups with 9–11 genes [26].

Hox gene has a consensus element with 180 bps, and its sequence encodes a DNA binding globular domain. Based on the evolutionary tree, *Hox* genes can be classified into 11 classes (i.e., ANTP, PRD, LIM, POU, HF, SINE, TALE, CUT, PROS, ZF, and CERS), containing approximately 250 *Hox* genes in humans [26]. The increasing numbers of *Hox* genes are due to two extra rounds of genome duplication, and subsequent loss of paralogs has been discovered in humans [24]. The *Hox* genes are involved in various processes ranging from the earliest stages of development–embryonic stem cells [27]–to patterning (particularly the *Hox* genes). Notably, mutations in the *Hox* genes cause developmental defects. An increasing number of studies have demonstrated the aberrant expression of *Hox* genes in tumorigenesis, suggesting that –besides developmental regulation–these genes play a critical role in the generation of cancer. In this review, we will focus on the role of the *Hox* genes in the generation of HCC, including tumor-initiating stem-like cells (TICs), epithelial to mesenchymal transition (EMT), immunotolerance, and viral infection, and list the promoting or repressing function of *Hox* genes in HCC (Table1-2).

Table1. Homeobox genes suppress HCC progression

Homeobox gene	Experiment model	Function in HCC	ref
Aristaless-Like Homeobox-4 (ALX4)	cell lines	Overexpression of ALX4 inhibits the proliferation, invasion, and EMT.	[28]
Barx homeobox 1 (*Barx1*)	cell lines mouse model HCC tissues	**1.** Low expression of Barx1 correlates with poor prognosis. **2.** Barx1 suppresses invasion and metastasis by inhibiting *MGAT5* and *MMP9* transcription,	[29]
Barx homeobox 2 (*Barx2*)	HCC tissues	Low expression of BARX2 is correlated with tumor metastasis.	[30]
caudal-type homeobox 1 (CDX1)	HCC tissues	Low expression of CDX1 is associated with poor prognosis,	[31]
arrest-specific homeobox (Gax)	cell lines HCC tissues	Gax expression inhibits NF-kappa B signal, and its expression negatively regulated by miR-301a.	[32]

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hematopoietically expressed homeobox protein (Hhex)	cell lines mouse model HCC tissues	1. Overexpression of Hhex resulted in decreases expression of c-Jun and Bcl2, and increases expression of P53 and Rb. 2. Hhex expression attenuates tumorigenicity in nude mice.	[33]
Homeobox D10 (HOXD10)	cell lines	HOXD10 is downregulated by miR-224 repression that cause cell migration and invasion.	[34]
Nk2 homeobox 8(Nkx2.8)	cell lines HCC tissues	Nkx2.8 expression is downregulated in HCC, and low Nkx2.8 expression negatively correlated with poor survival in patients	[35]
NK3 homeobox 1 (*NKX3.1*)	cell lines mouse model HCC tissues	NKX3.1 suppresses tumor proliferation and invasion by up-regulating Foxo1 expression.	[36]
paired related homeobox 1 (PRRX1)	cell lines HCC tissues	1. Hepatic cancer-stem cell properties are disrupted by PRRX1 overexpression 2. PRRX1 overexpression induces HCC apoptosis Via the p53- signaling	[37,38]

Table2. Homeobox genes promote HCC progression

Homeobox gene	Experiment model	Function in HCC	ref
caudal-related homeobox 2 (CDX2)	cell lines	CDX2 binds to CDH17 promoter and modulates its expression.	[39]
Distal-less homeobox 2 (DLX2)	cell lines HCC tissues	1. Overexpression of DLX2, in HCC tissues is an indicator of poor prognosis.	[40]

		2. DLX2 increases sorafenib resistance by promoting the ERK pathway and EMT.	
Distal-less 4 (DLX4)	cell lines HCC tissues	1. DLX4 is up-regulated in HCC tissues. 2. miR-122 binds 3'UTR of DLX4 for down-regulated its expression.	[41]
goosecoid (GSC)	cell lines HCC tissues	GSC expression is associated with metastasis and EMT in patients.	[42]
Homeobox HB9(HLXB9)	cell lines HCC tissues	HLXB9 upregulation is occurred in poorly differentiated HCC with a pseudoglandular pattern.	[43]
HOXA13	cell lines HCC tissues	1. HOXA13 expression is associated with tumor size, microvascular invasion, pathological grade, tumor capsula status, AFP level, metastasis and microvessel density. 2. Overexpression of HOXA13 increases colony formation on soft agar and migration, and reduces sensitivity to sorafenib.	[44,45]
Homeobox B7 (HOXB7)	cell lines mouse model HCC tissues	1. High HOXB7 expression is associated with larger tumor size and higher rate of biliary invasion. 2. HOXB7 promotes c-Myc and Slug expression through the AKT activation resulting HCC progression. 3. HOXB7 promotes proliferation, migration, and	[46-48]

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		invasion through activation of MAPK/ERK axis.	
Homeobox B9 (HOXB9)	cell lines HCC tissues	HOXB9 regulates TGF-β1 and ZEB1 signaling to promote EMT and cancer metastasis.	[49,50]
Iroquois homeobox (IRX)	cell lines	IRX3 induces proliferation, migration and invasion, but its expression is repressed by miR-377.	[51]
Intestine-Specific Homeobox (*ISX*)	cell lines mouse model HCC tissues	1. ISX is a regulator in HCC progression as a prognostic and therapeutic target in HCC. 2. Cyclin D1 and E2F1 are downstream genes of ISX in HCC 3. ISX involves kynurenine-AHR axis and immunosuppression effect of PD-L1 and CTLA-4 for immune escape by HCC	[52-54]
NANOG	cell lines mouse model HCC tissues	1. NANOG expression is required for TICs of HCC. 2. Nanog maintains TICs through the Insulin-Like Growth Factor Pathway in HCC. 3. Nanog promotes EMT through Stat3-dependent Snail activation. 4. HCV-NS5A induces TLR4-NANOG axis Promote Formation of liver TICs.	[55-64]
POU class 5 homeobox 1 (POU5F1 or OCT4)	cell lines mouse model HCC tissues	OCT4 expression is required for TICs.	[64-66]

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Pre-B-Cell Leukemia Homeobox 3 (*PBX3*)	cell lines Chick model HCC tissues	miR-33a-3p suppresses the cell growth, spreading and invasion by inhibiting *PBX3* expression.	[67]
Prospero-related homeobox 1 (Prox1)	cell lines mouse model HCC tissues	1. PROX1 is required for hepatocyte migration. 2. High PROX1 expression is associated with poor survival and tumor recurrence of HCC. 3. PROX1 promotes HCC Metastasis by induction and stabilization of HIF1a. 4. MAZ contributes to Prox1 isoform expressions in HCC. 5. PROX1 positively regulate HCC proliferation and sorafenib resistance by enhancing β-catenin signaling	[68-70]
short stature homeobox 2 (SHOX2)	cell lines HCC tissues	SHOX2 gene is associated with poor prognosis	[71]
sineoculis homeobox homolog 1 (*SIX1*)	cell lines HCC tissues	The expression status of SIX1 is associated with the 5-year survival rate duration of patients with early stage (I-II) of HCC, but not advanced stage (III-IV) of HCC	[72]
Zinc finger E-box binding homeobox 1/2 (ZEB1/2)	cell lines mouse model HCC tissues	ZEB1/2 is a transcription factor as a hub that promotes tumor invasion and metastasis by inducing EMT (detail in the text)	[73-94]

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Hox genes in TICs of HCC

In *Hox* genes, octamer-binding transcription factor 4 (*Oct4*) and *Nanog* reportedly control the self-renewal and pluripotency of pluripotent stem cells. Moreover, both of them were identified as critical non-cell-surface markers in TICs of various types of tumors. Several cell-surface antigens, including PROM1 (CD133), THY1 (CD90), epithelial cell adhesion molecule (EpCAM), and CD24 have been discovered on liver TICs. Increasing evidence indicates that liver TICs play a critical role in hepatocarcinogenesis [95]. Therefore, it is essential to investigate the mechanism through which the *Hox* genes are regulated in TICs. The elucidation of this mechanism may assist treating physicians in achieving improved clinical outcomes for patients with HCC. *MiR-429* has been identified as an oncogene in HCC by promoting the self-renewal, tumorigenicity, and chemoresistance of EpCAM -positive TICs. *MiR-429* promotes the transcription of *Oct4* by reducing the expression of retinoblastoma-binding protein 4, which otherwise inhibits the *E2F1* transactivation of *Oct4* transcription [65]. TICs expressing *Nanog* differentiate into mature cancer cells *in vivo* and *in vitro*. These cells are characterized by high capacity for tumor invasion and metastasis and are resistant to treatment with sorafenib and cisplatin. These TICs are required for *Nanog* to promote the expression of insulin-like growth factor (IGF) 2 and IGF 1 receptor (IGF1R) for self-renewal [59], and the NODAL/SMAD3 signaling pathway for tumor invasion [60]. It has been reported that EMT-associated transcription factors Snail and Slug or the androgen/androgen receptor axis directly regulate the expression of *Nanog* under transforming growth factor-β (TGF-β) signaling in TICs [55,56,61]. Interestingly, chromatin immunoprecipitation-sequencing analyses of *Nanog* showed that it regulates the expression of mitochondrial metabolic genes required in TICs. Moreover, it represses mitochondrial oxidative phosphorylation genes to prevent the reactive oxygen species (ROS) induction and induce oxidation of fatty acid for self-renewal and drug resistance of TICs [63]. Nevertheless, in murine nonalcoholic steatohepatitis model, *Nanog* contribute to the reprogramming of hepatic progenitor cells in driving these cells to TICs [57].

Hox in EMT of HCC

EMT, is converted processes of adherent epithelial cells into migratory mesenchymal cells that admit the cell invade the extracellular matrix, is normally required for gastrulation and neural crest migration during development. However, it is also involved in the early stages of tumor transformation into aggressive malignancies by increasing the potential for invasiveness and metastasis. Typically, cells undergoing EMT are characterized by decreased levels of E-cadherin, increased levels of N-cadherin and vimentin, and translocation of β-catenin from the membrane to the nucleus. An increasing number of studies showed that *Hox* genes can positively or negatively regulate EMT in HCC.

Zinc finger E-box binding Hox 1 (ZEB1) and ZEB2 are master transcription factors positive regulating invasion and metastasis by promoting EMT in cancer cells. Both ZEB1 and ZEB2 comprise two zinc finger domains at N-and C-terminals and the Smad interaction domain, homeodomain, CtBP interaction domain at the central region. In breast cancer, ZEB1 and ZEB2 have been shown to directly bind to the E-box located in the E-cadherin promoter [96], recruiting the CtBP transcriptional co-repressors [97] and/or the SWI/SNF chromatin remodeling protein BRG1 [98]. This results in repression of E-cadherin expression and promotion of EMT. In HCC patients, ZEB1 expression has been associated with low expression of E-cadherin, venous

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invasion, and TNM stage. Patients expressing high levels of ZEB1 and low levels of E-cadherin are associated with poorer prognosis [75].

In recent years, several regulations have been identified upstream of ZEB1 or ZEB2. Depletion of thrombomodulin–a natural anticoagulation factor–induces HCC cell migration by induction of ZEB1 and reduction of E-cadherin [73]. Moreover, 14-3-3e–a protein belonging to the highly conserved in eukaryotic cells 14-3-3 protein family–suppresses the expression of E-cadherin via regulation of ZEB1. It has been shown that this regulation increases the risk of metastasis and decreases the survival rates in HCC patients [74]. Previous studies have shown that MYC-associated zinc finger protein (MAZ) promotes the expression of *c-Myc*, *Ras*, *VEGF*, and podoplanin and represses that of *p53*, *Sp4*, and endothelial nitric oxide synthase in tumor development. MAZ was also shown to promote EMT and metastasis in HCC patients [82]. Depletion of the minus-end-directed motor protein kinesin family member C1 [84]–also termed HSET–downregulates ZEB1 to reduce EMT in HCC. Numerous studies have shown that mutations of the liver kinase B1 cause cancer (i.e., lung, mammary gland, ovarian, melanoma, and HCC). In addition, it is involved in liver carcinogenesis by promoting ZEB1 expression-associated EMT [86]. Forkhead box Q1, a forkhead transcription factor, induces EMT in HCC by up-regulated the expression of ZEB2 [89].

Regulatory proteins can manipulate the levels of ZEB1 and ZEB2 in EMT. However, there are numerous microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) which participate in ZEB1-mediated EMT. For example, miR-139-5p–which suppresses EMT in HCC by binding to ZEB1 and ZEB2 mRNA–was downregulated in HCC tissue [91]. In addition, MiR-101 binds to the 3′-untranslated region of ZEB1 to silence and disrupt the EMT in HCC. However, this regulation can be reversed by snoRNA host gene 6 transcript acting as a competing endogenous RNA [78,81]. MiR-211-5p and miR-154 targeting ZEB2 suppress HCC metastasis and tumor growth [90,94]. *Gα12 gep* is an oncogene that generates G-protein-coupled receptors sensing the increasing levels of ligands in tumor microenvironments. The activated *Gα12* promotes EMT through the induction of ZEB1 and represses p53-responsive miRNAs (i.e., miR-192, miR-215, and miR-200a), which target ZEB1 and ZEB2 [77,87]. Long non-coding RNA activated by TGF-β (lncRNA-ATB) promotes EMT under TGF-β stimulation by binding with the miR-200 family, which directly targets ZEB1 and ZEB2 mRNA [76]. Another lncRNA ZFAS1 also up-regulated the expression of ZEB1 by binding competitively to miR-150, consequently inducing EMT and invasion [79]. An increasing number of studies have shown that upstream antisense transcription controls the transcription of the corresponding genes [99]. Interestingly, a non-coding antisense transcript termed ZEB1 antisense 1 is generated from the promoters of ZEB1 from head-to-head conformation. ZEB1 antisense 1 promotes HCC invasion, metastasis, and EMT by targeting ZEB1 [80]. A similar regulation mechanism was also found between ZEB2 and ZEB2 antisense 1 [92]. Recent studies showed that lncRNAs competitively bind with miRNAs that maintain the expression of ZEB1 and EMT, including lncRNA MALAT1 and miR-143-3p, lncRNA TUG1, and Mir-142-3p [83,85].

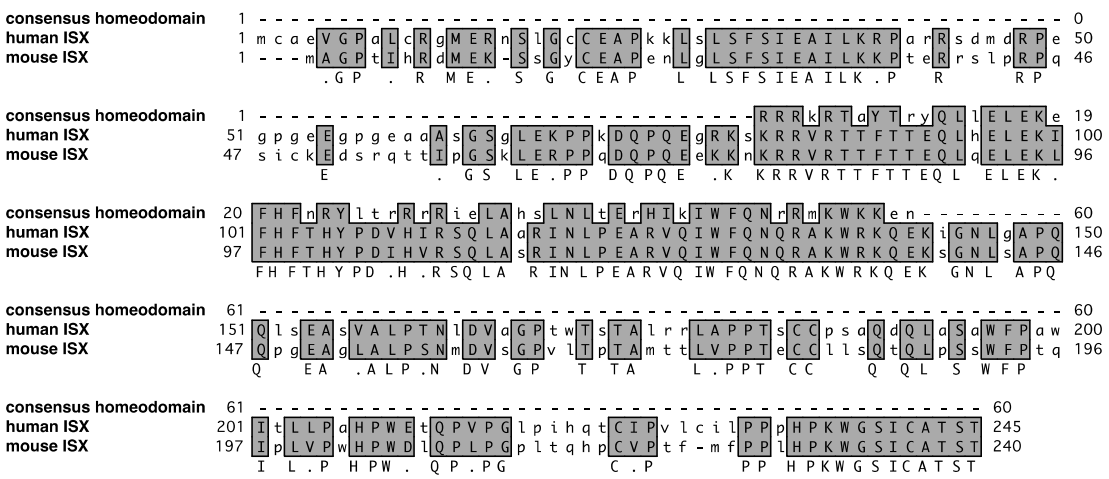
***Hox* genes and immunotolerance in HCC**

Liver sinusoids are involved in the central immunological functions of the liver [100]. With this system, liver has the capacity to remove many different microbes, microbe-associated molecules, and DAMPs, which continuously circulate from the gut to the liver. The diverse innate and adaptive immune cells residing in the liver to detect and clear blood-borne infectious organisms include Kupffer, natural killer, natural killer T, and

CD4+/8+ T cells. These immune cells respond to large and diverse cell-surface ligands expressed by infected, damaged, or transformed cells, leading to the changes in the innate and adaptive immune reactions through the production of different potent cytokines [101]. In addition, sinusoidal endothelial cells, stellate cells, and hepatocytes are associated with liver homeostasis and maintenance of balance between immunotolerance and immune activation. This prevents liver damage from non-pathological or continuous inflammatory stimuli and induces systemic immunotolerance [102]. Deregulation of the precisely regulation of immunological network promotes the development of HCC possibly due to chronic infection (e.g., infection with HBV or HCV), fat accumulation (i.e., NASH), or DAMPs derived from toxic liver damage (alcoholic liver disease) [103].

The rare reports focus on *Hox* gene and immune suppression in tumor cells. One example is that the intestine-specific Hox transcription factor (ISX), which has a consensus homeodomain (Fig.1), is a proto-oncogene involved in HCC development. Recently, our lab demonstrated that ISX involves in a positive feedback loop, including inflammation, tryptophan catabolism, and immune suppression. IL-6 induces the transcriptional activation of ISX to promote the production of tryptophan catabolic enzymes tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase 1 in HCC. Both enzymes increase the level of tryptophan catabolite,kynurenine, and aryl hydrocarbon receptor (AhR) and activate kynurenine/AHR axis. Its activation promotes a positive feedback mechanism to increase ISX associated proliferation, tumorigenesis, and immunotolerance. Apart from the AHR-dependent immunotolerance, overexpression of ISX induced level of genes encoding the immune modulators CD86 (B7-2) and PD-L1 and presented a repressed $CD8^+$ T-cell response [53].

Figure1. The alignment identify the consensus homeodomain of ISX



Hox genes in HBV- and HCV-associated HCC

Clinical and epidemiological studies have linked chronic hepatic inflammation with pathogenesis of HCC [104]. The chronic HBV and HCV-induced inflammation contributes to the development of HCC [105]. Notably, this tumorigenesis was associated with the regulation of numerous *Hox* genes. The Hox-A13 protein is induced in liver stem-like cells and HBV or HCV-infected HCC, but not in hepatocytes and bile duct epithelia [106]. The function of the prospero-related Hox protein 1 in the progression of HCC is debatable. However, it represses HBV antigen expression and genome replication through repression of the enhancer II/core promoter, preS1 promoter, and enhancer I/X promoter of HBV [107]. In HBV-infected HCC cells, IL-6 increases IGF1R and resulting the stemness related properties that evaluate the Oct4/Nanog that confers poor prognosis [66].

Furthermore, the HBx encoded by the HBV X gene, has been shown the association in the HCC development. The HBX with C-terminally truncation causes more malignant HCC by promoting metastasis and tumorigenicity. The reason is that HBx-ΔC1 is involved in the regulation of the properties of liver cancer stem cells by up-regulating the expression of *Hox* and *NANOG* through the stat3 pathway [108]. The regulation of HCV by Hox is promoting TICs. Overexpression of HCV non-structural protein NS5A and induction Toll-like receptor 4 (*TLR4*) by alcohol-induced endotoxemia in hepatocyte synergistically generate liver damage and tumor development, resulting in the generation of *Nanog*-positive TICs [62,109]. Of note, *TLR4*/*Nanog*-dependent TICs are also found in HCC patients. Research has identified a *TLR4*/*Nanog*-mediated activation of YAP1 and IGF2BP3, which are novel molecules responsible for the inhibition of the TGF-β pathway and the development of chemoresistance [110]. Using a high-cholesterol/high-fat diet, it was shown that a higher proportion of hepatocyte-specific NS5A transgenic mice developed liver tumors containing TICs. This was attributed to the activation of *TLR4-Nanog* and pSTAT3 signaling pathways through an exaggerated EMT via the induction of *Twist1 [62]*.

Perspectives and future directions in new HCC adjuvant regiments: a link between chronic inflammation, immune system and hepatocarcinogenesis

It has been reported that the majority of HCC patients occur in relation to a cirrhotic liver, i.e., the final stage of hepatic fibrosis [111]. Fibrosis occurs when the repeated and continuous liver injuries through various etiologies, such as viral hepatitis B and C, alcoholism, and NASH. Studies have shown that liver injury in viral hepatitis is caused by the viral protein-mediated host immune and inflammation response but not the result of direct cytopathic effects of the viruses [112,113]. In fact, hepatic fibrosis comprises a series of cellular events and the production of a variety of cytokines, aiming to orchestrate chronic inflammation [114]. Damage to hepatocytes will trigger the release of ROS and fibrosis-related mediators. This subsequently induces response from the hepatic stellate cells, phagocytic Kupffer cells, liver sinusoidal endothelial cells, and the extracellular matrix. These steps are important in the development of fibrosis [115]. In addition, several cytokines, including platelet-derived growth factor [116], TGF-β [117], TNF-α [118], interferon, and ILs [119,120], are involved in hepatic fibrosis. Conversely, Caldwell *et al.* stated that 5%–30% of patients with HCC may not have a readily identifiable risk factor [13]. It has been noted that the majority of "cryptogenic" HCC cases in the USA result from NAFLD, which is a spectrum of disorder ranging from simple steatosis to NASH and cirrhosis. Obesity, particularly abdominal obesity, is found in 30%–100% of subjects with NAFLD. Steatosis is 4.6-fold higher in obese individuals than in those who with normal weight [121,122]. Moreover, it was supported by the clinical, epidemiological, and biochemical data that NAFLD is the hepatic manifestation of the metabolic syndrome in association with resistance to the effects of insulin on peripheral glucose and utilization of fatty acids [123]. Importantly, obesity is considered a status of chronic inflammation and strongly linked to an increased incidence of HCC [124]. In obesity, the enlargement of white adipose tissue induces endoplasmic reticulum stress, leading to subsequent release of free fatty acids and inflammatory cytokines. The recruitment of immune cells to white adipose tissue further enhances local and systemic inflammation [125].

Chronic inflammation contributes to the development of HCC through several proposed routes. Chronic inflammation increases oxidative stress, which may induce mutations of DNA and lead to HCC. In addition, it may induce increased hepatocytes proliferation, telomeres shortening, and consequent chromosomal instability and intended malignant alterations [126]. Various aberrant genomic mutations have been identified in the

chronic hepatic inflammation and considered the drivers of HCC development and progression. These alterations include mutations interfering telomere maintenance, activation of the Wnt pathway, inactivation of p53, chromatin remodeling, Ras signaling, mTOR signaling, and initiation of the ROS pathway.

Numerous proinflammatory cytokines alter the liver microenvironment during hepatic fibrosis, as well as initiate and promote hepatocarcinogenesis [127]. For example, TNF-α–a master regulator of liver homeostasis–mediates the balance between the proliferation and apoptosis of hepatocytes. The TNF receptor 1 (TNFR1) participates in the activation of the proinflammatory nuclear factor κB and mitogen-activated protein kinase pathways. Liver-resident Kupffer cells and recruited macrophages are considered the main sources of TNF-α. Compensatory proliferation of hepatocytes is the key step for tumor initiation. The recruited macrophages secrete TNF-α to block hepatocyte apoptosis, which is toward survival and proliferation of hepatocyte. Permanent proliferating hepatocytes cause DNA damage to accumulate and result in generation of more mutations, which promote development of HCC [128]. Conversely, genetic mouse models revealed that TNFR1 inactivation deescalate the development of liver tumors [129].

It was found that TNFR1 deficiency mice fed with high-fat diet suppressed of obesity- enhanced HCC, as well as attenuated hepatosteatosis, which was induced by obesity [130]. IL-6–an important signaling molecule in response to infections and systemic inflammation–is induced by other inflammatory cytokines (e.g., TNF-α, IL-1, adipokines). Up-regulated IL-6 levels strongly correlate with an increased risk of HCC progression and *vice versa*. This observation was also noted in patients suffering from HCC [131,132]. Angiogenesis has been an important step in the development of HCC. IL-6 trans-signaling promotes HCC through inhibition of p53-induced apoptosis and enhances angiogenesis by up-regulating the proliferation of endothelial cells and tumor development [133]. Moreover, IL-6 induced STAT3 signaling also activates the hypoxia-inducible factor 1-α and VEGF [134]. Moreover, IL-6 is involved in the activation of the PI3K/AKT/mTOR signaling cascade. Notably, aberrant activation of mTOR signaling is observed in 50% of human HCC cases [135]. Collectively, this evidence demonstrates that IL-6 signaling plays a pivotal role in the development and progression of HCC.

Conclusions

Dysregulated expression of HOX genes are wildly identified in different aspects of HCC development, including HBV and HCV infection, TICs, EMT, and immunotolerance. HOX genes are discovered in both positive and negative regulation in HCC progression; however, HOX-associated regulatory networks in HCC remain unclear. It is noteworthy that integrating single cell RNA sequencing of different cell types in HCC to identify the role of Hox genes in regulation of tumor microenvironment, TICs, or other unknown functions and build a systemic interaction network of HOX gene in HCC. It can provide more information for the detailed mechanism of the cross regulation of HOX genes and for the design of more efficacious therapy of HCC patients.

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