Table 3. HBV status in HBsAg-negative recipients who experienced HBV reactivation after hematopoietic stem cell transplantation (HSCT) and their donors.

Recipients (Before HSCT)			Recipients (After HSCT)								Donor	
Case	anti-HBs	anti-HBc	Last Confirmed Time of HBsAg-Negativity after HSCT (Months)	Time of HBsAg-Positivity after HSCT (Months)	HBV DNA at the Time of HBsAg-Positive (log copies/mL)	Type of NUCs	Period from HBsAg Positive to NUCs Start (Months)	Outcome of HBV Status	Period from Treatment Start to Achievement of HBV Outcome (Months)	Treatment Period (Months)	anti-HBs	anti-HBc
1	NA	NA	2	8	(+)	ND	NA	HBsAg (−)	NA	NA	NA	NA
2	NA	NA	1	18	(+)	ND	NA	HBsAg (+)	19 *	NA	NA	NA
3	NA	NA	0	33	8.2	ETV	141	HBsAg (+)	12	12	(−)	(−)
4	NA	NA	0	10	(+)	ND	NA	NA	NA	NA	(−)	(−)
5	NA	NA	0	19	7.4	LAM	24	HBsAg (+)	1	1	NA	NA
6	(+)	(+)	10	28	4.5	LAM	0	anti-HBs (+)	8	>8	NA	NA
7	(+)	NA	0	31	7.3	LAM	0	anti-HBs (+)	48	9	NA	NA
8	(+)	(−)	0	4	4	LAM	0	anti-HBs (+)	11	>11	(−)	(−)
9	(+)	(+)	5	14	8.9	ETV	41	HBsAg (+)	10	>10	(−)	(−)
10	(+)	(+)	3	20	>9	ETV	4	HBsAg (+)	6	>6	(−)	(−)
11	(+)	(+)	74	91	8.6	ETV	0	HBsAg (−)	15	>15	(−)	(−)

HSCT, hematopoietic stem cell transplantation; NUCs, nucleos(t)ide analogues; anti-HBs, antibody to hepatitis B surface antigen; anti-HBc, antibody to hepatitis B core antigen; (+), positive; (−), negative; NA, not available; ND, not done; LAM, lamivudine; ETV, entecavir.; * Period from detection of HBsAg-positive to HBV outcome.

3. Discussion

In the present study, the HBV reactivation rate was 100% in HBsAg-positive recipients with HSCT in the absence of HBV prophylaxis. In HBsAg-negative recipients with HSCT, 2.7% of the recipients experienced HBV reactivation. HBV reactivation was observed in 11% of HBsAg-negative and anti-HBc-positive recipients, although the number of study samples was relatively small. Notably, all recipients with HBV reactivation were successfully treated with NUCs, although some needed long-term treatment after HSCT. To add support to this conclusion, further extended follow-up periods will be needed.

In HBsAg-positive recipients, the prophylactic use of NUCs was effective for preventing HBV reactivation, supporting previous reports [13–16]. In the case of HBsAg-positive recipients, NUCs seem effective when administered with allogeneic [14] as well as with autologous HSCT [9,13]. In the present study, the 5 HBsAg-positive cases could not become NUCs-free during the follow-up periods. All patients died of primary diseases after receiving NUCs for a median period of 15 months (2–224 months). Lin *et al.* [17] reported fatal fulminant hepatitis B cases after withdrawal of prophylactic lamivudine in HSCT. Extended NUCs therapy may be safe and effective for the prevention of HBV reactivation in HBsAg-positive recipients with HSCT [18].

In HBsAg-negative recipients, we found that HBV reactivation was a rare event in both anti-HBc-negative and anti-HBs-negative recipients with HSCT. HBV reactivation occurred in almost all cases with anti-HBc and/or anti-HBs, except one case whose anti-HBc/anti-HBs status was unknown (Table 1). The status of anti-HBc and anti-HBs as well as HBsAg should be confirmed before performing HSCT, as in previous reports [19,20]. Goyama *et al.* [19] reported that the use of corticosteroids, the lack of anti-HBs in donor, and a decrease in serum anti-HBc and anti-HBs levels may predict reverse seroconversion after HSCT. Of note, HBV reactivation occurred in 82% of HBsAg-negative cases during immunosuppressive treatment in the present study. Some recent guidelines [21–23] have been recommended to start prophylactic antiviral therapy for HBsAg-negative recipients with anti-HBc and receiving HSCT. Tomblyn *et al.* [22] reported that, if the HSCT recipient is anti-HBc-positive and anti-HBs-positive, the risk of HBV reactivation is considered low during chemotherapy/conditioning, but it is thought to be higher following prolonged treatment with prednisone for graft-*versus*-host disease. They [22] also recommended that prophylactic antiviral treatment may be considered for anti-HBc-positive and anti-HBs-positive recipients before, and for 1 to 6 months after HSCT.

It is known that HBV vaccine could induce anti-HBs in a majority of vaccinees [24]. Because there are no universal vaccination programs against HBV in Japan, HBV infections are still viewed as an important issue [25]. Then, we should consider recipients with anti-HBs as having experienced HBV infection, perhaps a setting different from other countries where universal vaccination programs against HBV exist. HBV immunization of recipients of allogeneic HSCT results in a protective antibody response against HBV [26], and further studies concerning this issue are urgently required in our country.

Hui *et al.* [27] reported that it is uncertain whether late HBV-related hepatitis is due to *de novo* hepatitis B infection or transmission from donors. In our study, at least 6 of 11 donors for HBsAg-negative-recipients with HBV reactivation did not have anti-HBs and/or anti-HBc (Table 3). As

for the frequency of follow-up of these recipients, most of them did not receive HBsAg/anti-HBs checks regularly after HSCT, or regular follow-up. As recent Japanese guidelines recommended that recipients with HBsAg, anti-HBs, or anti-HBc should receive HBsAg/anti-HBs examinations regularly after HSCT, or regular follow-up [28], we plan to perform monitoring of HBV DNA monthly for 12 months after HSCT, and monthly once per 3 months after that.

In the present study, we did not observe recipients with fatal severe liver diseases. This might be because we used NUCs in the early stage of HBV reactivation. In HBsAg-negative recipients who are anti-HBc-positive and/or anti-HBs-positive, close monitoring including the measurement of HBV DNA as well as ALT levels should be mandatory. Although the intervals of this monitoring may be discussed, the present study suggested that immediate use of NUCs might be safe and effective for the prevention of HBV reactivation in HBsAg-negative recipients with HSCT.

4. Patients and Methods

4.1. Ethics

This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This retrospective study was approved by the Ethics Committee of Chiba University, Graduate School of Medicine on 31 January 2014 (No. 1754). Informed consent for participation in this study was obtained from all patients and/or their families by posting a notice in our institutes.

4.2. Patients

A total of 413 recipients (mean observation period, 25 (0–309) months), treated with HSCT at Chiba University Hospital, Chiba, Japan between June 1986 and March 2013, were retrospectively reviewed for the occurrence of HBV reactivation. The patient characteristics are shown in Table 4. The recipients were divided into two groups: recipients treated with HSCT from 1986 to 1999 (before 2000), and recipients treated with HSCT from 2000 to 2013 (in 2000 or after), as nucleic acid amplification testing (NAT) of donated blood for infectious agents was introduced in Japan in October 1999, and Japanese health insurance approved the first NUC, lamivudine, for the treatment of hepatitis B recipients in September 2000.

Table 4. Patient characteristics in the present study.

Characteristics	Total (n = 413)	1986–1999 (n = 120)	2000–2013 (n = 293)
Gender			
Male, n (%)	250 (60.5)	70 (58.3)	180 (61.4)
Female, n (%)	163 (39.5)	50 (41.7)	113 (38.6)
Median age, years (range)	42 (15–69)	32 (15–57)	47 (16–69)
Type of disease, n (%)			
AML	115 (27.8)	36 (30.0)	79 (26.7)
ALL	61 (14.8)	28 (23.3)	31 (10.6)
CML	38 (9.2)	20 (16.7)	18 (6.1)
MDS	31 (7.5)	9 (7.5)	22 (7.5)

Table 4. *Cont.*

Characteristics	Total (n = 413)	1986–1999 (n = 120)	2000–2013 (n = 293)
Lymphoma	84 (20.3)	18 (15.0)	68 (23.2)
Plasma cell dyscrasia *[1]	61 (14.8)	0 (0)	61 (20.8)
Aplastic anemia	16 (3.9)	9 (7.5)	7 (2.4)
Others *[2]	7 (1.7)	0 (0)	7 (2.4)
Transplant type, n (%)			
Autologous	114 (27.6)	15 (12.5)	99 (33.8)
Allogeneic	299 (72.4)	105 (87.5)	194 (66.2)
For Allogeneic-SCT Donor source, n (%)			
Related	126 (42.1%)	59 (56.2%)	67 (34.5%)
Unrelated	135 (45.2%)	46 (43.8%)	89 (45.9%)
Unrelated cord blood	38 (12.7%)	0 (0%)	38 (19.6%)

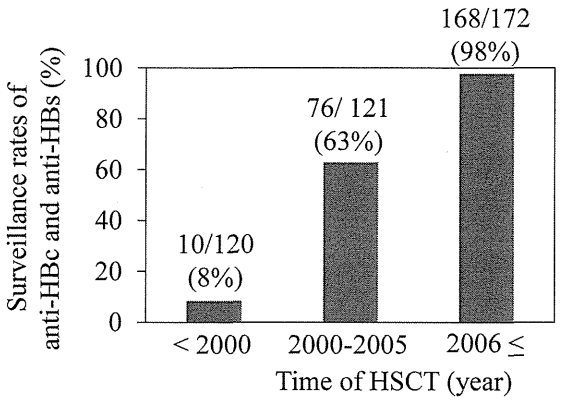
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; SCT, stem cell transplantation. *[1] Plasma cell dyscrasia included multiple myeloma, AL amyloidosis and POEMS syndrome; *[2] Others included primary myelofibrosis, chronic active EB virus infection and chronic eosinophilic leukemia.

4.3. Serological Examination

HBsAg, anti-HBs and anti-HBc were determined by ELISA or chemiluminescent enzyme immunoassay (CLEIA) [29]. Depending on the time-point during this retrospective study, HBV DNA was measured by Roche Amplicore PCR assay (detection limit: 2.6 log IU/mL), COBAS TaqMan HBV test v2.0 (detection limit: 2.0 log IU/mL) (Roche Diagnostics, Basel, Switzerland), or PCR methods. If needed, we performed in-house nested PCR [29]. All serological tests were performed at the Central Laboratory of Chiba University Hospital.

HBsAg was measured in all recipients before and after HSCT. Among 120 recipients with HSCT before 2000, anti-HBs and anti-HBc were measured in only 10 (8%) recipients. Among the patients with HSCT performed in 2000 or after, the recipients were further divided into two groups: 2000–2005 and 2006–2013. Of 121 recipients with HSCT in 2000–2005, anti-HBs and anti-HBc were measured in 76 (63%) recipients. Finally, among 172 recipients with HSCT in 2006–2013, anti-HBs and anti-HBc were measured in 168 (98%) recipients (Figure 1). HBsAg, anti-HBs and anti-HBc were measured for screening in almost all recipients after the Japanese Health and Labor Sciences Research Group for "Clarification of current status for reactivation of hepatitis B virus associated with immunosuppressants and antineoplastics and establishment of preventive measures" started a registry in 2009 for HBV-infected patients with hematopoietic malignancies [28].

Figure 1. Surveillance rates of antibodies to hepatitis B core antigen (anti-HBc) and to hepatitis B surface antigen (anti-HBs) according to the time of hematopoietic stem cell transplantation (HSCT).



4.4. Definition of HBV Infection Status and HBV Reactivation

In the present study, before HSCT, the recipients were divided into two groups according to HBsAg: HBsAg-positive and HBsAg-negative. Among the HBsAg-negative recipients, anti-HBc-positive and/or anti-HBs-positive recipients were considered as having experienced previous HBV infection [2], and recipients without anti-HBc and anti-HBs were considered as having no previous or current HBV infection.

We defined ≥1 log IU/mL increase in serum HBV DNA from nadir as HBV reactivation in HBsAg-positive recipients. We also defined ≥1 log IU/mL increase, or the re-appearance of HBV DNA from baseline and/or HBsAg, as HBV reactivation in HBsAg-negative recipients.

4.5. Statistical Analysis

Statistical analyses were performed using Statview-J 5.0 (SAS institute, Cary, NC, USA). HBV reactivation rates were calculated by Kaplan-Meier method and evaluated by log-rank test. Baseline was taken as the date of HSCT. $p < 0.05$ was considered statistically significant.

5. Conclusions

HBV reactivation was a common event in HBsAg-positive recipients with HSCT for hematologic malignancies, NUCs are safely and effectively used in these recipients, and extended NUCs therapy may be needed for the prevention of HBV reactivation. In addition, HBV reactivation was occasionally observed in HBsAg-negative recipients with anti-HBc and/or anti-HBs and treated with HSCT, and the immediate use of NUCs could prevent the progression to severe liver damage. Special attention should be paid to recipients with previous exposure to HBV.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (9): Hepatitis B virus

Regulation of microRNA by hepatitis B virus infection and their possible association with control of innate immunity

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Abstract

Hepatitis B virus (HBV) chronically infects more than 350 million people worldwide. HBV causes acute and chronic hepatitis, and is one of the major causes of cirrhosis and hepatocellular carcinoma. There exist complex interactions between HBV and the immune system including adaptive and innate immunity. Toll-like receptors (TLRs) and TLR-signaling pathways are important parts of the innate immune response in HBV infections. It is well known that TLR-ligands could suppress HBV replication and that TLRs play important roles in anti-viral defense. Previous immunological studies demonstrated that HBV e antigen (HBeAg) is more efficient at eliciting T-cell tolerance, including production of specific cytokines IL-2 and interferon gamma, than HBV core antigen. HBeAg downregulates cytokine production in hepatocytes by the inhibition of MAPK or NF-κB activation through the interaction with receptor-interacting serine/threonine protein kinase. MicroRNAs (miRNAs) are also able to regulate various biological processes such as the innate immune response. When the expressions of approximately 1000 miRNAs were compared between human hepatoma cells HepG2 and HepG2.2.15, which could produce HBV virion that infects chimpanzees, using real-time RT-PCR, we observed several different expression levels in miRNAs related to TLRs. Although we and others have shown that HBV modulates the host immune response, several of the miRNAs seem to be involved in the TLR signaling pathways. The possibility that alteration of these miRNAs during HBV infection might play a critical role in innate immunity against HBV infection should be considered. This article is intended to comprehensively review the association between HBV and innate immunity, and to discuss the role of miRNAs in the innate immune response to HBV infection.

Key words: Hepatitis B virus; HepG2.2.15; Innate immunity; MicroRNA; Persistent infection; Toll-like receptor

Core tip: Hepatitis B virus (HBV) is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma in the world. HBV could interact with the host's innate and adaptive immune responses to establish chronic infection. HBV also interacts with Toll-like receptors (TLRs) and TLR signaling pathways, and regulates host immune responses through the regulation of microRNAs (miRNAs) to some extent. This article fo-

cuses on the involvement of miRNA in the association between HBV and TLR signaling pathways and reviews the miRNAs involved in HBV infection.

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INTRODUCTION

Hepatitis B virus (HBV), a member of hepadona viridae, has partially circular double-stranded DNA genome, 3.2 kb in length[1]. It contains four overlapping open reading frames that encode seven proteins: the precore protein, also known serologically as HBe antigen (HBeAg), the core protein (HBcAg), viral polymerase, three forms of the envelope protein known as S antigen (HBsAg) and X (HBx) protein[1,2]. HBV as well as hepatitis C virus (HCV) causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC)[3]. Hepatic cirrhosis and HCC are the most common causes of death in patients with chronic liver disease[4].

The outcome of HBV infection is the result of complex interactions between HBV and the immune system including adaptive and innate immunity[5,6]. Toll-like receptors (TLRs) are important parts of the innate immune response in hepatitis virus infections[7]. There are several reports about the important role of TLRs and TLR-mediated signaling in the pathogenesis and outcome of HBV infection[2,5-11].

MicroRNA (miRNA) is one of the endogenous noncoding small RNAs, approximately 18-22 nucleotides in size, a post-transcriptional regulator that binds to the 3'-untranslated region (UTR) of the target gene messenger RNA, usually resulting in cleavage or inhibiting translation of the target gene mRNA[12,13]. It is estimated that the human genome may encode over 2000 miRNAs, which may control about 60% of the human genome[14,15]. Physiologically, miRNAs are able to regulate various biological processes such as cell proliferation, differentiation and apoptosis, neuroprocesses, carcinogenesis and immune response[16-18]. This article is intended to comprehensively review the association between HBV and innate immunity, and to discuss the role of miRNAs in the innate immune response to HBV infection.

INNATE IMMUNITY IS IMPORTANT FOR THE ERADICATION OF HBV

Interferons (IFNs) play an important role in the innate immune response to virus infection. IFN-α and IFN-β (type I IFNs) are secreted by almost all virus-infected cells including hepatocytes and by specialized blood lymphocytes. In contrast, the production of IFN-γ (type II IFN) is restricted to cells of the immune system, such as natural killer (NK) cells, macrophages, and T cells. On the other hand, tumor necrosis factor alpha (TNF-α) primarily initiates innate immune response and triggers acquired immune responses[19]. TNF-α-induced apoptosis is important for clearance of hepatocytes infected with HBV and HCV, and IFN-γ accelerates the killing of theses hepatocytes[19,20]. The previous studies demonstrated that TNF-α and IFN-γ downregulate HBV gene expression in the liver of HBV transgenic mice by post-transcriptionally destabilizing the viral mRNA[21-23]. It has been widely believed that the cytotoxic T lymphocyte response clears viral infections by killing infected cells. However, Chisari's group[21-24] reported that noncytopathic clearance of HBV from hepatocytes by cytokines, which abolish viral replication and HBV gene expression, is another important mechanism. Isogawa *et al*[24] reported that TLR3, TLR4, TLR5, TLR7 and TLR9 ligands could induce antiviral cytokines and inhibit HBV replication in HBV transgenic mice, thereby indicating TLR activation as a powerful strategy for the treatment of chronic HBV infection. HBV replication can be controlled by innate immune response, involving TLRs, if it is activated in hepatocytes[24]. Together, these facts indicate that innate immunity including TLR signaling plays an important role in the pathogenesis of HBV infection.

TOLL-LIKE RECEPTORS AND ANTI-VIRAL DEFENSES

TLRs, germline-encoded pattern recognition receptors (PRRs), can play a central role in host cell recognition and response to various pathogens such as viruses[25]. TLR1, TLR2, TLR4, TLR5, and TLR6 are expressed on the cell surface while TLR3, TLR7, TLR8 and TLR9 are expressed within intracellular vesicles. TLR3, TLR7/8 and TLR9 are involved in the recognition of viral nucleotides such as double-stranded RNA, single-stranded RNA and DNA, respectively[26]. Other than TLRs, membrane-bound C-type lectin receptors (CLRs), cytosolic proteins such as NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs), which include retinoic acid-inducible gene I (RIG- I), melanoma differentiation antigen 5 (MDA5), and lipophosphoglycan biosynthetic protein 2 (LPG2), and unidentified proteins that mediate sensing of cytosolic DNA or retrovirus infection, are also involved in the recognition of pathogen-associated molecular patterns (PAMPs)[25].

TLRs play a crucial role in defending against pathogenic infection through the induction of inflammatory cytokines and type I IFNs by myeloid differentiation primary response 88 (MYD88)-dependent and MYD88-independent pathway. In the MYD88-dependent pathway, MYD88 recruits a set of signal cascades such as MAPK and NF-κB through receptor-interacting serine/

threonine protein kinase (RIPK/RIP). In the MYD88-independent pathway, TLR3 activates NF-κB and MAPKs through RIPK. TLR3 also activates IFN regulatory factor 3 (IRF3) and IRF7 *via* TRIF/TICAM-1, inducing the production of type I IFN. The activated NF-κB and IRFs are translocated to the nucleus. NF-κB and MAPKs initiate the transcription of inflammatory cytokine genes, whereas IRFs initiate the transcription of type I IFN[2]. RIG- I and MDA5 pathways can also activate IRF3 to produce type I IFNs. RNA helicases RIG- I and MDA5, specific receptors for double-stranded RNA, and the downstream mitochondrial effector known as CARDIF/MAVS/VISA/IPS-1, are also major pathways for type I IFN induction.

ASSOCIATION BETWEEN HBV AND TOLL-LIKE RECEPTORS

TLRs have been recognized as playing an important role in the pathogenesis of chronic hepatitis B[8]. NF-κB is activated by three TLR adaptors, MYD88, Toll/interleukin (IL)-1 receptor (TIR)-domain-containing adaptor-inducing IFNβ (TRIF), and IFN promoter stimulator 1 (IPS-1), to elicit anti-HBV response in both HepG2 and Huh7 cells[27]. Down-regulations of TLR7 and TLR9 mRNA were observed in peripheral blood mononuclear cells (PBMC) of HBV-infected patients[28]. Chen *et al*[29] reported that TLR1, TLR2, TLR4 and TLR6 transcripts were also downregulated in PBMC of chronic hepatitis B patients. After being challenged by TLR2 and TLR4 ligands, cytokine production was impaired in PBMC of chronic hepatitis B patients on the basis of the levels of plasma HBsAg[29]. Xie *et al*[30] reported that HBV infection results in reduced frequency of circulating plasmacytoid dendritic cells (pDCs) and their functional impairment *via* inhibiting TLR9 expression. HBV replication suppresses the TLR-stimulated expression of pro-inflammatory cytokines (TNF, IL6) and the activation of IRF3[31]. It has also been reported that HBV could target RIG- I signaling by HBx-mediated IPS-1 down-regulation, thereby attenuating the antiviral response of the innate immune system[32].

HBV E ANTIGEN DOWNREGULATES CYTOKINE PRODUCTION

The HBV precore/core region of HBV genome also encodes HBeAg as well as the HBV core. The precore stop codon prevents the formation of precore protein and HBeAg[2,33]. The existence of HBeAg in serum is known to be a marker of a high degree of viral infectivity. In Japan, the major HBV genotypes are B and C, but our previous study[34] revealed that the precore mutation A1896 and the core promoter mutations at nt1762 and 1764 were found more frequently in acute liver failure than in acute hepatitis, and HBV genotype B was predominant in acute liver failure. It has also been shown that acute liver failure occasionally occurs in persons who are negative for HBeAg[35,36]. It is well known that perinatal transmission of HBV occurs in about 10%-20% of HBeAg-negative mothers without prevention of perinatal HBV transmission by combined passive and active immunoprophylaxis, and the babies are at risk of developing fulminant hepatitis[37]. Chronic hepatitis B with high HBV DNA and ant-HBe is associated with a severe and evolutive liver disease[38]. These clinical findings could be assumed to have immune tolerance for HBeAg, although the function of HBV precore or HBeAg is unknown. Previous immunological studies[39-41] demonstrated that HBeAg is more efficient at eliciting T-cell tolerance, including production of its specific cytokines IL-2 and IFN-γ, than HBV core antigen. We also demonstrated that HBeAg expression inhibits IFN and cytokine production[2] and that HBeAg physically associates with RIPK2 and regulates IL-6 gene expression[6]. Visvanathan *et al*[42] reported that the expression of TLR2 on hepatocytes, Kupffer cells, and peripheral monocytes was significantly reduced in HBeAg-positive chronic hepatitis B patients. Thus, HBV seems to have evolved strategies that block the effector mechanisms induced through IFN and/or cytokine signaling pathways, similar to other viruses[19].

MIRNAS WERE DIFFERENTIALLY EXPRESSED IN HEPG2.2.15 AND HEPG2

HepG2.2.15 cells assemble and secrete HBV virion that infects chimpanzees[43,44]. We examined the expression of approximately 1000 miRNAs in the human hepatoma cells HepG2.2.15 and HepG2 using real-time RT-PCR, the most sensitive technique for mRNA detection and quantification[45,46].

First, 1008 miRNAs were examined in the hepatoma cells HepG2.2.15 and HepG2, using quantitative real-time RT-PCR with specific primers (Qiagen, Hilden, Germany). SNORD61, SNORD68, SNORD72, SNORD95, SNORD96A and RNU6-2 were used as endogenous controls to normalize expression to determine the fold-change in miRNA expression between the test sample (HepG2.2.15) and control sample (HepG2) by 2-ddCT (comparative cycle threshold) method[21]. MiRNAs were annotated by Entrez Gene (NCBI, Bethesda, MD, United States), accessed on 2/27/2013. Data were analyzed with miRNA PCR array data analysis software (http://www.sabiosciences.com/mirnaArrayDataAnalysis.php). Scatter plot analysis is shown in Figure 1A. There were differences in expression between HepG2 and HepG2.2.15 (Figure 1B).

We then excluded 599 miRNAs according to the following criteria: (1) average threshold cycle was relatively high (> 30) in either HepG2 or HepG2.2.15, and was reasonably low in the other samples (< 30); (2) average threshold cycle was relatively high (> 30), meaning that its relative expression level was low, in both HepG2 and

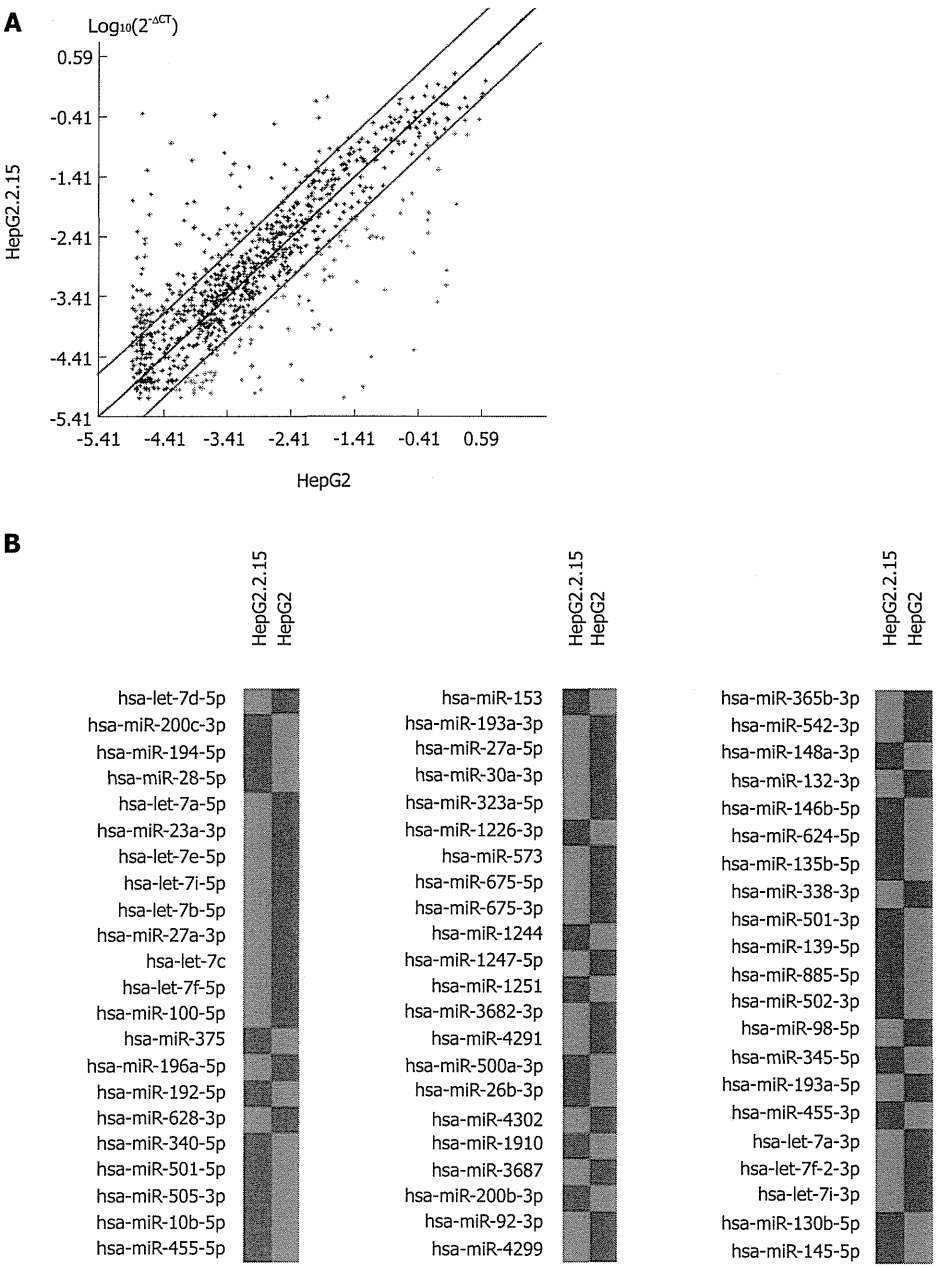

Figure 1 MicroRNAs expression in hepatoma cells HepG2.2.15 and HepG2. A: Scatter plots of 1008 miRNAs indicate 2^{-dCT} numerical values in HepG2 cells (x-axis) and HepG2.2.15 cells (y-axis). The black line indicates fold changes of 1. The pink lines indicate 5-fold change in miRNA expression threshold, comparing HepG2.2.15 with HepG2. Red + indicates miRNA expressed at least 5-fold higher in HepG2.2.15 than in HepG2 cells. Green + indicates miRNA expressed at least 5-fold lower in HepG2.2.15 than in HepG2 cells. Black + indicates that the difference of miRNA between the two cells was within 5-fold; B: Comparison of miRNAs expression between HepG2 and HepG2.2.15 cells. Red color indicates miRNA expressed at least 5-fold higher in HepG2.2.15 than in HepG2 cells. Green color indicates miRNA expressed at least 5-fold lower in HepG2.2.15 than in HepG2 cells. miRNAs: MicroRNAs.

HepG2.2.15; and (3) average threshold cycle was either not determined or was greater than the defined cut-off value (default 35) in both samples, meaning that its expression was undetected, making this fold-change result erroneous and uninterpretable.

Out of 409 miRNAs examined, 30 (7.3%) were upregulated by 5-fold or greater in HepG2.2.15 compared to HepG2. Twelve miRNAs (miR-200b-3p, miR-505-3p, miR-148a-3p, miR-145-5p, miR-194-5p, miR-885-5p, miR-192-5p, miR-146b-5p, miR-340-5p, miR-375, miR-139-5p and miR-200c-3p) were upregulated 10-fold or more in HepG2.2.15 cells. MiRNAs upregulated 5-fold or more are shown in Figures 1B and 2A. On the other hand, out of 409 miRNAs, 35 (8.6%) were downregulated 5-fold or more in HepG2.2.15 compared to HepG2. Twenty-two miRNAs (let-7c, miR-573, let-7b-5p, miR-338-3p, miR-100-5p, miR-92b-3p, miR-542-3p, miR-4302, miR-4291, miR-193a-5p, miR-98-5p, miR-4299, miR-132-3p, let-7f-2-3p, let-7f-5p, let-7i-5p, let-7d-5p, miR-193a-3p, let-7a-5p, let-7i-3p, miR-196a-p and let-7a-3p) were downregulated 10-fold or more in HepG2.2.15 cells. MiRNAs downregulated 5-fold or

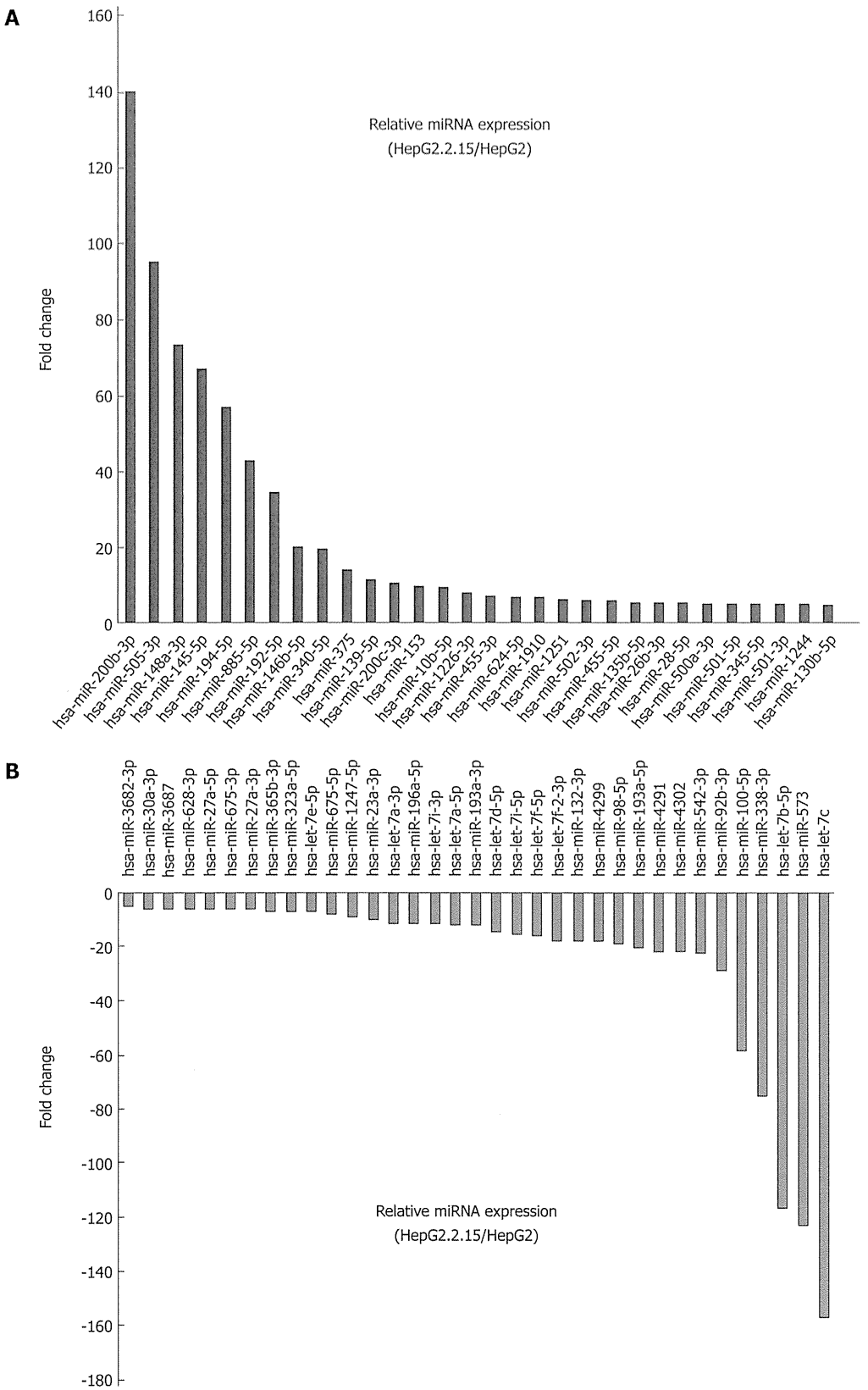

Figure 2 MicroRNAs expressed at more than 5-fold difference between hepatoma cells. HepG2.2.15 and HepG2 cells. A: MiRNA expressed at least 5-fold higher in HepG2.2.15 than in HepG2 cells; B: MiRNA expressed at least 5-fold lower in HepG2.2.15 than in HepG2 cells. miRNAs: MicroRNAs.

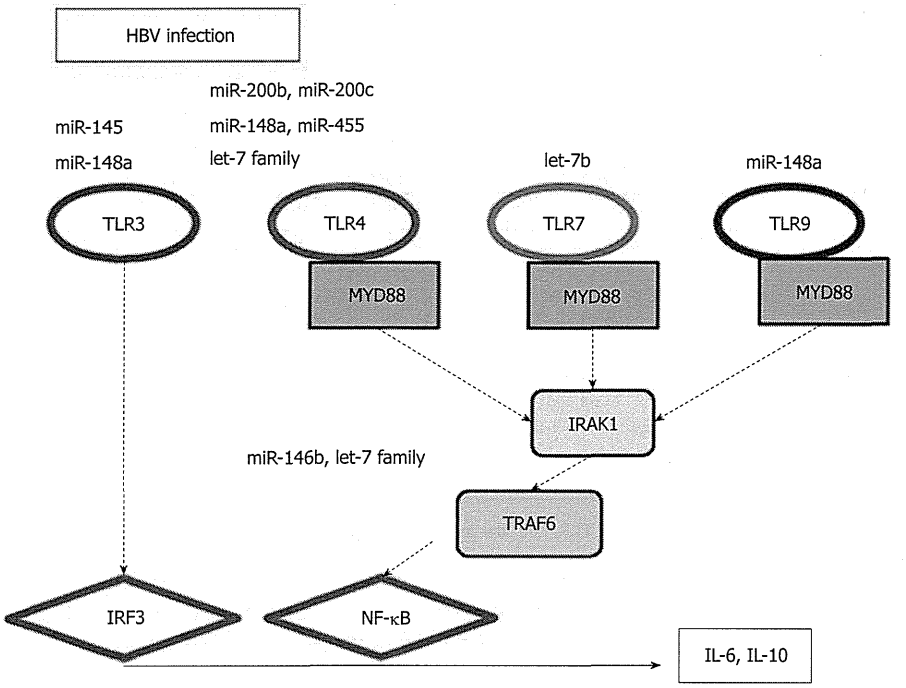

Figure 3 MicroRNAs and Toll-like receptor signaling pathway in hepatitis B virus infection. IRAK: Interleukin (IL)-1 receptor-associated kinase 1; IRF: Interferon regulator factor; miRNA: MicroRNA; MYD88: Myeloid differentiation factor 88; NF-κB: Nuclear factor-κB; TLR: Toll-like receptor; TRAF: Tumor necrosis factor receptor-associated factor.

Table 1 MicroRNAs associated with Toll-like receptor signaling pathways, upregulated by 5-fold or greater in HepG2.2.15 than in HepG2

MicroRNAs	Genomic location	Fold changes	Description of target molecules/pathways	Ref.
miR-200b-3p	1p36.33	140.15	TLR4 signaling through MyD88-dependent pathway	[47]
miR-148a-3p	7p15.2	73.36	TLR3, TLR4 and TLR9 agonists upregulated miR-148/152 expression	[48]
miR-145-5p	5q32	66.97	miR-145 promoted interferon-β induction by SOCS7	[49]
miR-146b-5p	10q24.32	20.05	TNF receptor-associated factor 6 and IL-1 receptor-associated kinase 1	[50]
miR-200c-3p	12p13.31	10.75	TLR4 signaling through MyD88-dependent pathway	[47]
miR-455-3p	9q32	7.36	miR-455 was involved in TLR4 signaling pathway through E2F1 transcription factor	[51]
miR-455-5p	9q32	5.76	miR-455 was involved in TLR4 signaling pathway through E2F1 transcription factor	[51]

Genomic location was analyzed using GeneCards (http://gene4.weizmann.ac.il/). TLR: Toll-like receptor.

more are shown in Figures 1B and 2B.

MIRNAS RELATED TO TLR PATHWAY UPREGULATED IN HEPG2.2.15 CELL LINES

Innate immunity represents the first line of defense against HBV, and we and others have reported its importance in the persistence of HBV infection[2,5-11]. So, we focused on miRNAs related to the TLR pathway. Among miRNAs upregulated 5-fold or more in HepG2.2.15 cells, 7 miRNAs (miR-200b-3p, miR-148a-3p, miR-145-5p, miR-146b-5p, miR-200c-3p, miR-455-3p and miR-455-5p) were reported to be related to TLR pathways (Table 1). MiRNAs miR-200b and miR-200c are the factors that modify the efficiency of TLR4 signaling through MYD88 in HEK293 cells[47]. TLR3, TLR4 and TLR9 agonists upregulated miR-148/152 expression and downregulated calcium/calmodulin-dependent protein kinase II (CaMK II) in dendritic cells (DCs) on maturation[48]. Thus miR-148/152 can act as fine-tuners in regulating the innate response and antigen-presenting capacity of DCs[48]. Exogenous miR-145 promoted IFN-β induction by targeting the suppressor of cytokine signaling 7 (SOCS7), through the nuclear translocation of signal transducer and activator of transcription 3 (STAT3) and SOCS7-silencing enhanced IFN-γ induction by stimulation with TLR3 ligand, poly(I-C)[49]. MiR-146 plays a role in the control of TLR and cytokine signaling through a negative feedback regulation loop involving down-regulation of interleukin (IL)-1 receptor-associated kinase 1 (IRAK1) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) protein levels[50]. MiR-455 was involved in the TLR4 signaling pathway through E2F1 transcription factor[51].

Table 2 MicroRNAs associated with Toll-like receptor signaling pathways, downregulated by 5-fold or greater in HepG2.2.15 than in HepG2

MicroRNAs	Genomic location	Fold changes	Description of target molecules/pathways	Ref.
let-7e-5p	19q13.33	-7.29	Akt1 activated by TLR4-ligand LPS, positively regulated let-7e	[52]
let-7a-3p	9q22.32 11q24.1 22q13.31	-11.44	Repression of let-7 family relieves IL-6 and IL-10 mRNAs from negative post-transcriptional control in TLR4 signaling pathway	[53]
let-7i-3p	12q14.1	-11.57	let-7i regulates Toll-like receptor 4 expression	[54]
let-7a-5p	9q22.32 11q24.1 22q13.31	-11.96	Repression of let-7 family relieves IL-6 and IL-10 mRNAs from negative post-transcriptional control in TLR4 signaling pathway	[53]
let-7d-5p	9q22.32	-14.03	Repression of let-7 family relieves IL-6 and IL-10 mRNAs from negative post-transcriptional control in TLR4 signaling pathway	[53]
let-7i-5p	12q14.1	-15.10	let-7i regulates Toll-like receptor 4 expression	[54,55]
miR-132-3p	17p13.3	-18.18	TNF receptor-associated factor 6 and IL-1 receptor-associated kinase 1	[56]
let-7b-5p	22q13.31	-116.31	let-7b activates TLR 7	[56]

Genomic location was analyzed using GeneCards (http://gene4.weizmann.ac.il/). TLR: Toll-like receptor.

MIRNAS RELATED TO TLR PATHWAY DOWNREGULATED IN HEPG2.2.15 CELL LINES

Among miRNAs downregulated 5-fold or more in HepG2.2.15 cells, 8 miRNAs (let-7e-5p, let-7a-3p, let-7i-3p, let-7a-5p, let-7d-5p, let-7i-5p, miR-132-3p and let-7b-5p) were reported to be related to TLR pathways (Table 2). Protein kinase Akt1, which is activated by the TLR4-ligand lipopolysaccharide (LPS), positively regulated let-7e and miR-181c but negatively regulated miR-155 and miR-125b[52]. Repression of the let-7 family relieves IL-6 and IL-10 mRNAs from negative post-transcriptional control in the TLR4 signaling pathway[53], and the miRNAs let-7i and let-7b activate TLR4 and TLR7, respectively[54-56].

ROLE OF MIRNAS IN REGULATION OF INNATE IMMUNE RESPONSE IN HBV INFECTION

In the present study, 30 and 35 miRNAs were upregulated and downregulated, respectively, by 5-fold or greater in HepG2.2.15 compared to its parental cell line HepG2. These results indicate that miRNAs could play an important role in chronic persistent HBV infection. Su *et al*[57] reported that miR-155 enhances innate antiviral immunity through promoting the JAK/STAT signaling pathway by targeting SOCS1, inhibiting HBV replication. The possibility cannot be ruled out that HBV persistently infects hepatocytes through the regulation of miRNAs.

We also speculated that several of the miRNAs involved in the TLR signaling pathway play a critical role in innate immunity against HBV infection[5,24] (Figure 3). It has been reported that miR-21[58], miR-22[59,60], miR-122[58], miR-194[61] and miR-219-1[62] are associated with chronic persistent HBV infection as well as its clearance. In the present study, miR-194 was upregulated 10-fold or more in HepG2.2.15 cells.

CONCLUSION

MicroRNAs miR-122 and miR-130a play an important role in chronic hepatitis C[63,64]. Regulation of miRNAs also plays an important role in HIV infection[65]. In HCV infection, a set of miRNAs that regulate host immune response are modulated[66]. We and others have demonstrated that HBV modulates the host immune response. It might be possible that HBV as well as HCV regulates host immune response through the regulation of miRNAs in some steps toward chronic infection. MiRNAs and their regulation play a critical role in HBV infection, and HBV may regulate the TLR signaling pathway through the regulation of miRNAs.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (1): Hepatocellular carcinoma

Androgen receptor signaling in hepatocellular carcinoma and pancreatic cancers

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Abstract

Hepatocellular carcinoma (HCC) and pancreatic cancer remain difficult to treat, and despite the ongoing development of new treatments, the overall survival rate has only modestly improved over the past decade. Liver and pancreatic progenitors commonly develop from endoderm cells in the embryonic foregut. A previous study showed that HCC and pancreatic cancer cell lines variably express androgen receptor (AR), and these cancers and the surrounding tissues also express AR. AR is a ligand-dependent transcription factor that belongs to the nuclear receptor superfamily. Androgen response element is present in regulatory elements on the AR-responsive target genes, such as transforming growth factor beta-1 (TGF beta-1) and vascular endothelial growth factor (VEGF). It is well known that the activation of AR is associated with human carcinogenesis in prostate cancer as well as HCC and pancreatic cancer and that GRP78, TGF beta, and VEGF all play important roles in carcinogenesis and cancer development in these cancers. HCC is a male-dominant cancer irrespective of its etiology. Previous work has reported that vertebrae forkhead box A 1/2 are involved in estrogen receptors and/or AR signaling pathways, which may contribute to the gender differences observed with HCC. Our recent work also showed that AR has a critical role in pancreatic cancer development, despite pancreatic cancer not being a male dominant cancer. Aryl hydrocarbon (or dioxin) receptor is also involved in both HCC and pancreatic cancer through the formation of complex with AR. It is possible that AR might be involved in their carcinogenesis through major histocompatibility complex class I chain-related gene A/B. This review article describes AR and its role in HCC and pancreatic cancer and suggests that more specific AR signaling-inhibitors may be useful in the treatment of these "difficult to treat" cancers.

Key words: Androgen receptor; Gender difference; Hepatocellular carcinoma; Male-dominant; Pancreas

Core tip: Recent studies have shown that androgen receptor (AR) could play an important role in carcinogenesis and cancer development in hepatocellular carcinoma (HCC) and pancreatic cancer. HCC is a male-dominant cancer. Although pancreatic cancer is not male-dominant, because liver and pancreatic progenitors develop commonly from endoderm cells in the embryonic foregut, AR might play an important role in these cancers.

Kanda T, Jiang X, Yokosuka O. Androgen receptor signaling in hepatocellular carcinoma and pancreatic cancers. *World J Gastroenterol* 2014; 20(28): 9229-9236 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i28/9229.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i28.9229

INTRODUCTION

The liver and pancreas progenitors develop from endo-

derm cells in the embryonic foregut[1]. The liver arises from lateral domains of endoderm in the developing ventral foregut[1,2], and from a small group of endodermal cells tracking down the ventral midline[1-3]. The pancreas is also induced in lateral endoderm domains, adjacent and caudal to the lateral liver domains, and in cells near the dorsal midline of the foregut[4,5]. Under specific experimental conditions, pancreatic progenitor(s) and pancreatic oval cells may differentiate into hepatocytes[1]. Thus, given that the liver and pancreas share differentiation patterns[6], it is possible that carcinogenesis and cancer development in the liver and pancreas may resemble each other.

The occurrence of hepatocellular carcinoma (HCC) has increased in Japan[7] and is increasing in the United States[8]. Cirrhosis due to chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections is the leading risk factor for HCC[9,10]. HCC is a male-dominant cancer[11]. Advanced HCC, defined as metastatic or locally advanced disease not responsive to locoregional therapies such as surgery[12], local ablation[13], or transcatheter arterial chemoembolization[14], is still associated with poor prognosis even if anti-angiogenesis therapies, especially treatment with sorafenib, were used for treatment[15].

The global annual incidence rate of pancreatic cancer is reported to be approximately 8/100000 persons and pancreatic cancer has an exceptionally high mortality rate[16,17]. Pancreatectomy seems to offer the only chance for long-term survival[18], although recent advancement in chemotherapies brought the improvement of survival of patients with invasive cancer in Japan[19]. These facts indicate the urgent need for the development of novel and more effective adjuvant therapies for HCC and pancreatic cancer.

ANDROGEN RECEPTOR

Androgen and androgen receptor (AR) signaling has an important role in the initiation and progression of many hormone-related cancers including prostate and breast cancer[20]. AR consists of the N terminus harboring transcriptional activation domain(s), a central DNA-binding domain and a C-terminal ligand-binding domain. AR is a testosterone/5-alpha-dihydrotestosterone (DHT)-dependent transcription factor belonging to the nuclear receptor superfamily[21,22]. AR is primarily responsible for mediating the physiological effects of androgens by binding to specific DNA sequences, androgen responsive element (ARE), which is present in regulatory elements on the AR-responsive target genes. Androgen binding changes the protein conformation in AR and leads to its translocation from the cytoplasm into the nucleus. In the nucleus, AR forms a homodimer and is recruited to the ARE. ARE is present in regulatory elements on the AR-responsive target genes, such as transforming growth factor beta-1 (TGF beta-1)[23] and vascular endothelial growth factor (VEGF)[24]. Glucose-regulated protein 78 (GRP78/BiP), one of the endoplasmic reticulum chaperones, is also an AR-interacting protein[25]. Thus, the transcriptional activation function of AR is associated with gender differences as well as human carcinogenesis.

AR AND HCC

Expression of AR in HCC tissues

It has been reported that AR is expressed in HCC and the surrounding liver tissues[26,27]. An immunohistochemical study showed that 67.7% of HCC were positive for AR; 51.6% for estrogen receptor (ER); 83.9% for progesterone receptor (PgR) and 38.7% for apolipoprotein D receptor and that chronic HCV infection correlated positively with AR and PgR status[28]. Some HCC specimens have elevated AR, ER and PgR expression, indicating that these hormone receptors could be involved in hepatocarcinogenesis and cancer development. The recurrence rate of HCC was significantly higher in the AR-positive group than in the AR-negative group, and the survival rate of HCC was better in AR-negative patients than in AR-positive patients[29]. Boix *et al*[30] reported that higher tumor recurrence was observed in AR-positive surrounding tissues than in AR-negative tissues. There were also contrary reports[30-32]. Only two-thirds of HCC contained AR, and there seemed no clear association between AR expression and liver fibrosis[30]; thus, further investigation might be needed.

AR and HBV

More than 2 billion people have been exposed to HBV and 350 million are chronically infected with HBV globally. HBV causes acute and chronic hepatitis, cirrhosis, and HCC[33,34]. We compared the gene expression profile for nuclear receptors and related genes between HepG2.2.15, which secretes complete HBV virion, and HepG2 by real-time RT-PCR[35]. Among AR-related molecules, nuclear receptor subfamily 1, group I, member 3 (NR1I3), mediator complex subunit 16 (THRAP5), mediator complex subunit 4 (MED4), mediator complex subunit 17 (CRSP6), mediator complex subunit 24 (THRAP4), mediator complex subunit 13 (THRAP1) and mediator complex subunit 1 (PPARBP) all were upregulated significantly higher in HepG2.2.15 cells compared to HepG2 cells[35]. These data support the association between HBV and AR[35,36]. It has been reported in Taiwan[37-40] that the association between the trinucleotide (CAG) repeats in the AR gene and higher testosterone levels increase in HCC risk. Higher levels of androgen signaling, reflected by higher testosterone levels and 20 or fewer AR-CAG repeats, which is the association of CAG repeat in the AR gene, might be associated with an increased risk of HBV-related HCC in men[37]. The CAG polymorphism in exon 1 of the AR gene has been associated with the development of HCC with shorter AR alleles conferring a higher risk in men[37,39,40]. In contrast, women harboring 2 AR alleles with more than 23 CAG repeats had an increased risk of HCC compared to women with only short alleles or a single long allele[38]. In women, hepatocytes expressing the longer AR allele seem to confer a higher risk for HCC[38,39]. Polymorphisms of

Table 1 Representative primary androgen-responsive genes and androgen-responsive genes lacking genomic androgen response elements, previously reported in hepatocllular carcinoma and/or pancreatic cancer

ARGs[24]	Signal pathways[1]	Reported diseases
Primary ARGs		
CYR61	Wnt/β-catenin	HCC, pancreatic cancer
SNAI2	Epithelial-mesenchymal transition (EMT)	HCC, pancreatic cancer
FN1	Cell surface and extracellular matrix	HCC
ATP1A1	Na,K-ATPase pump	Pancreatic cancer
FKBP5	AKT	Pancreatic cancer
VEGFA	Angiogenesis	HCC, pancreatic cancer
SGK	Serine/threonine kinase	HCC, pancreatic cancer
SLC22A3	Organic cation transporter	HCC, pancreatic cancer
NDRG1	P53/N-myc	HCC, pancreatic cancer
NFKBIA	NF-κB inhibitory family	HCC
PYGB	Glycogen catabolism	HCC, pancreatic cancer
ARGs lacking AREs		
ADAMTS1	Inflammatory process	HCC, pancreatic cancer
CXCR7	Orphan G-protein coupled receptor	HCC, pancreatic cancer
GATA2	Transcription factor	Pancreatic cancer
MYC	Oncogene	HCC, pancreatic cancer
KLF4	Cell proliferation	HCC, pancreatic cancer

[1]Genes were annotated in accordance with NCBI and Reference[24]. CYR61: Cystein-rich, angiogenic inducer, 61; SNAI2: Snail family zinc finger 2; FN1: Fibronectin 1; ATP1A1: ATPase, Na+/K+ transporting, alpha 1 polypeptide; FKBP5: FK506 binding protein 5; VEGFA: Vascular endothelial growth factor A; SGK: Serum/glucocorticoid regulated kinase 1; SLC22A3: Solute carrier family 22, member 3; NDRG1: N-myc downstream regulated 1; NFKBIA: Nuclear factor kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; PYGB: Phosphorylase, glycogen, brain; ADAMTS1: ADAM metallopeptidaas with thrombospondin type 1 motif, 1; CXCR7: Chemokine (C-X-C motif) receptor 7; GATA2: GATA binding protein 2; MYC: V-myc avian myelocytomatosis viral oncogene homolog; KLF4: Kruppel-like factor 4 (gut); ARE: Androgen responsive element.

the AR-regulating genes and cytokine genes might be related to HCC[41].

HBx protein is about 17 kDa in size, and associated with hepatocarcinogenesis[42,43]. HBx likely augmented AR activity by increasing the phosphorylation of AR through HBx-mediated activation of the c-Src kinase signaling pathway[44]. HBx can physically bind to the AR and increase the gene transactivation activity of AR[45,46]. HBx increased the N-terminal transactivation domain (NTD) activation of AR through c-Src kinase and also enhanced AR dimerization by inhibiting glycogen synthase kinase-3β activity, which acts as a negative regulator of the conformational changes of AR[46].

Further, AR promotes transcription of HBV, resulting in a higher HBV titer in male HBV carriers and an increased risk of HCC[47-49]. It was reported that higher HBsAg and HBV titers were found in male HBV transgenic mice, compared with control mice, and that HBV enhancer I contained a DNA element responsive to transcriptional regulation by ligand-stimulated AR[47]. Similar observations in HBV transgenic mice lacking AR in liver hepatocytes, were also reported by another group[48]. The absence of HBx did not affect the effects of gender, androgen, and AR on HBV replication[49]. The androgen pathway could increase the transcription of HBV through direct binding to the androgen-responsive element sites in HBV enhancer I[47]. The direct relationship between HBV and AR was supported by our HepG2 experiments[35].

AR and HCV

HCV infection affects approximately 4 million Americans and is the leading cause of cirrhosis and HCC in the United States[50]. HCV infection is a leading cause of HCC in Japan[7,11,51]. The HCV genome encodes structural proteins (core, E1, E2 and p7) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). The HCV core protein is approximately 21 kDa in size and is associated with hepatocarcinogenesis[52-57]. HCV augments AR-mediated signaling through the HCV core protein[58]. HCV infection and HCV core protein enhance AR activation in the presence of the AR-ligand DHT.

AR is activated by the MAPK, phosphatidylinositol 3-kinase/AKT and JAK/signal transducer and activator of transcription 3 (STAT3) pathways[59]. STAT3 is also involved in HCV-induced AR activation, and HCV augments the phosphorylation status of STAT3. HCV core increases STAT3 phosphorylation at both Ser-727 and Tyr-705, which in turn activates AR. AR expression increases HCV-mediated VEGF mRNA expression and angiogenesis[58]. The status of angiogenesis in HCC has been correlated with disease progression and prognosis. It has also been reported that higher serum testosterone is associated with increased risk of advanced hepatitis C-related liver diseases in men[60]. In immortalized human hepatocytes, HCV increased AR mRNA expression[58]. However, further studies are needed to determine whether AR increases HCV replication.

Androgen-responsive genes and androgen-responsive genes lacking genomic androgen responsive elements in HCC

The addition of functional AR in human HCC cells leads to the promotion of cell growth. AR may promote hepatocarcinogenesis via increased cellular oxidative stress and DNA damage as well as suppression of the p53-mediated DNA repairing system[61]. AR is also associated with HCC migration and invasion[62,63]. It has been reported that vertebrae forkhead box A 1/2 (Foxa1/2) is involved in estrogen receptor and/or AR signaling pathways, contributing to the observed gender differences of HCC[64]. Despite its cause, HCC is a male-dominant cancer; thus, AR could play a critical role in hepatocarcinogenesis and development. RNA expression profiling of LNCaP prostate cancer cells has described about 500 transcripts with altered expression[65,66]. Primary androgen-responsive genes (*ARGs*), that is, the subset regulated directly by AR-occupied androgen responsive elements (AREs), may in turn produce effects on secondary target genes[24]. Representative primary ARGs and ARGs lacking genomic AREs[24] that have been reported in HCC or pancreatic cancer are shown in Table 1. These genes have often

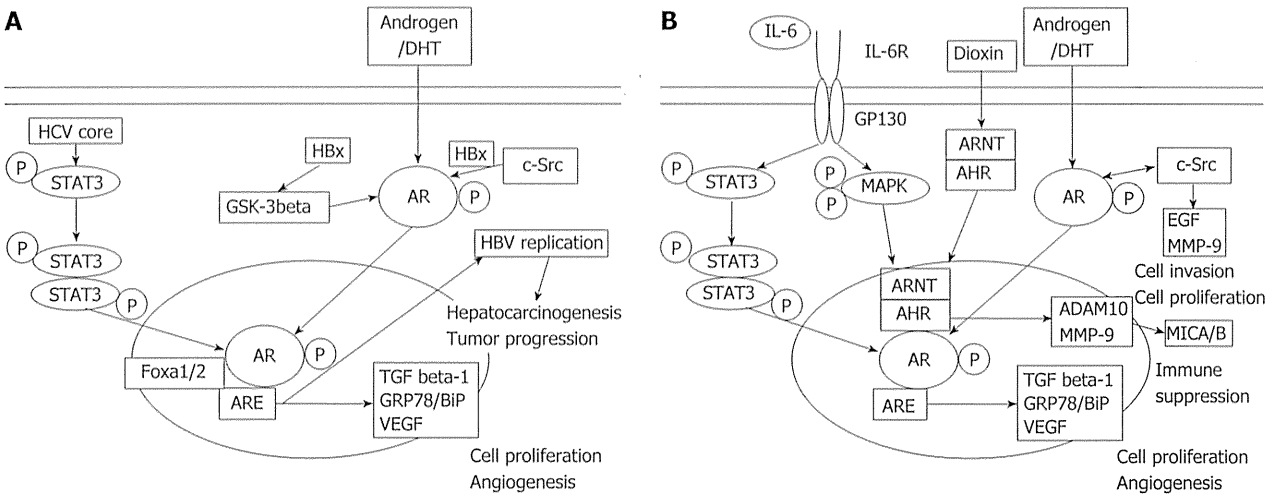

Figure 1 Androgen receptor signaling in hepatocellular carcinoma (A) and pancreatic cancers (B). ADAM10: A disintegrin and metalloprotease 10; AHR: Aryl hydrocarbon (or dioxin) receptor; AR: Androgen receptor; ARE: Androgen responsive element; ARNT: AHR nuclear translocator; DHT: 5-alpha-dihydrotestosterone; Foxa1/2: Vertebrae forkhead box A 1/2; GRP78/BiP: Glucose-regulated protein 78; GSK-3beta: Glycogen synthase kinase-3beta; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MICA/B: Major histocompatibility complex class I chain-related gene A/B; MMP-9: Matrix metalloprotease 9; STAT3: Signal transducer and activator of transcription 3; TGF beta-1: Transforming growth factor beta-1; VEGF: Vascular endothelial growth factor.

been reported to be associated with hepatocarcinogenesis and/or pancreatocarcinogenesis.

AR AND PANCREATIC CANCER

Human normal pancreas tissue and human pancreatic adenocarcinoma tissue also express AR[67]. Pancreatic cancer also over-expresses interleukin-6 (IL6), and serum IL6 levels are correlated with a poor prognosis of pancreatic cancer[68]. IL6 phospholylates MAPK and activates the AR NTD through STAT3[69,70]. MAPK and STAT3 increase the transactivation of AR[69,71]. We have demonstrated that IL6 increases the phosphorylation of STAT3 and MAPK, which in turn increases the activation of AR in pancreatic cancer cells[72]. IL6 also promotes pancreatic cancer cell migration in the presence of AR[72]. AR might play an important role in pancreatic carcinogenesis and the development of pancreatic cancer, making AR a candidate of therapeutic targets for new pancreatic cancer treatments[72,73].

Both aryl hydrocarbon (or dioxin) receptor (AHR) and AHR nuclear translocator (ARNT) are known to interact with AR in a testosterone-dependent manner[74,75]. AHR, but not ARNT, increased the AR-transcriptional activity independent of exogenous AHR ligand treatment [2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)][74]. It has also been shown that normal expression levels of ERK1/2 are required for testosterone to induce the interaction between AR and AHR and the expression of liver receptor homolog 1 in ovarian granulosa cells[75]. In humans, a wide variety of health effects and cancer development are associated with the exposure to dioxins[76]. Once the ligand is bound, AHR translocates into the nucleus, and the AHR-ARNT-dioxin complex then binds to dioxin response elements acting as transcriptional enhancers and induces the transcription of CYP1A1[77]. Previous work[78] has revealed that the liver and pancreas from AHR-deficient mice had no significant 2,3,7,8-TCDD-induced lesions. It has also been shown that AHR is involved in many physiological functions, including circadian rhythm[79], which is involved in both HCC[80-82] and pancreatic cancer[83,84]. AR might influence the progression of pancreatic cancer and HCC by affecting circadian rhythm, the disruption of which is associated with cancer development[84].

AHR also downregulates natural killer (NK) cell inflammatory cytokine production[85]. Several matrix metalloproteases (MMPs), such as a disintegrin and metalloprotease (ADAM)10 and MMP-9, are targets of AHR pathways[86]. ADAM10 and MMP-9 cleave NKG2D ligands, leading to the release of soluble major histocompatibility complex class I chain-related gene A (MICA) and MICB, which then act to inhibit NK cells, γδTCR bearing cells and some other T lymphocyte subsets, contributing to immune-suppression[79]. Interestingly, recent work has suggested that MICA is involved in human hepatocarcinogenesis[87,88] and that MICA/B is expressed in human HCC and hepatoma cell lines[89], despite their expression being significantly decreased in HCV-infected Huh7.5 cells[90].

The migration of tumor cells play a role in tumor progression and this process requires acquisition of an invasive phenotype involving MMPs production and cell motility[91]. Targeting the AR domain involved in AR/Src association impairs EGF signaling, which stimulates transmigration and MMP-9, in human fibrosarcoma HT1080 cells[92], suggesting AR might be new potential targets in the therapeutic approach to human pancreatic cancer. Further studies are needed.