Original Article

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IPS-1 polymorphisms in regulating interferon response in HBV infection

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Summary **Single nucleotide polymorphisms (SNP) influence the outcome of antiviral therapy in chronic hepatitis B patients. Interferon β promoter stimulator 1 polymorphisms (IPS-1) regulate interferon (IFN) mediated viral clearance in hepatitis B virus (HBV) infection. In our study, HepG2 and HepG2.2.15 were transfected with different SNP genotype expression vectors of IPS-1 (wild-type, rs17857295, rs7262903 and rs7269320). The production of IPS-1 and IFN were evaluated in these transfected cells. IPS-1 in the HepG2.2.15 cells transfected with rs17857295 or rs7262903 was 37% or 31% lower than that with wild-type transfection ($p < 0.001$). IFN-β in rs17857295 or rs7262903 transfected HepG2.2.15 cells was 5.4 or 3.7 fold higher than that of wild-type transfection ($p < 0.0001$). IPS-1 in rs7269320 SNP transfected HepG2.2.15 cells was 40% lower than that of wild-type transfection ($p < 0.0001$); no significantly different IFN-β was observed between rs7269320 SNP and wild-type transfections. IFN-β expression was > 2 fold higher in rs17857295 transfected HepG2.2.15 cells than HepG2 cells ($p < 0.001$). The data suggests that host HBV viral clearance is stronger in IPS-1 rs17857295 or rs7262903 SNP genotype patients than wild-type patients. Relatively weak inducible IFN-β production in HBV infected patients with IPS-1 rs7269320 SNP or wild-type may contribute to chronic virus infection.**

Keywords: IPS-1, single nucleotide polymorphism, hepatitis B virus, interferon response, chronic HBV infection

1. Introduction

Chronic hepatitis B (CHB) is still a major challenge to clinicians, due to its serious clinical complications with huge financial and psychological burdens (*1,2*). The precise pathogenesis of CHB is not fully clear, especially the host immunity in CHB viral clearance, and subsequent chronicity, despite decades of extensive research (*3*).

Single nucleotide polymorphism (SNP) is a polymorphism at the genome level, due to a single nucleotide missense mutation (*4*). Missense mutation of a single nucleotide in genetic code leads to the change of the sequence of bases, and transforms the amino acid sequence of the corresponding protein, influencing the structure and function of proteins. SNP is an important biological genetic marker, causing a biological genetic trait change. SNPs determine the effect of drug therapy and/or disease susceptibility in different genetic backgrounds, which may also contribute to different host immunities, especially antiviral immunity (*5*).

Host antiviral innate immunity is initiated *via* viral

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pathogen-associated molecular patterns (PAMP), such as viral double-stranded RNA(dsRNA). All RNA and most DNA viruses, including HBV (*6*), generate dsRNA molecules at the transcription or replication level. Retinoic acid inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) are very important pattern recognition receptors (PRRs) *in vivo* (*7*). RIG-I/MDA5 signaling pathway plays an important role in host recognition and clearance of the virus infection process (*8*). After binding to dsRNA, RIG-I transforms into an open conformation, translocates onto mitochondria, and interacts with the downstream adaptor to induce the production of interferon (IFN) and inflammatory factors *via* IRF3/7 and NF-κB pathways. IFN-β promoter stimulator 1 (IPS-1), an essential adaptor protein in the RIG-I/MDA5 anti-viral pathway, plays an important role in the cell recognition and the induce process of IFN-β during viral infection (*9-12*). Upregulation of IPS-1 results in increased production of IFN-β, which finally prevents viral entry (*9,12*). RIG-I mediated antiviral immunity and its downstream signaling pathway have been well illustrated in hepatitis C virus (HCV) infection (*13*). Following HBV stimulation, MDA5 activates innate immune signaling to suppress hepatitis B virus (HBV) replication (*14*). IFN inhibits HBV replication *via* the MDA5/IPS-1 pathway, but patients display different prognosis to HBV infection.

Studies on genetics determine susceptibility of HBV infection and subsequent chronicity has been well documented in families of segregation analysis or twin studies (*15,16*). SNP of IL28B is responsible for the therapeutic outcomes following PEG-IFN plus ribavirin treatment in chronic viral hepatitis C (CHC) (*17*). It has been demonstrated that three SNPs upstream of IL28B (rs12979860 CC, rs12980275 AA, and rs8099917 TT) are highly associated with HCV recovery, but inversely with HBV (*18*). Recent study of polymorphisms near IL28B (rs12980275AA, rs12979860 CC) have also demonstrated an association with serologic response to PEG-IFN in HBeAg+ CHB patients (*19*). Interestingly, IL28B rs12979860 CC genotype do not contribute to the outcome of HBV infection (*20*). HLA-DPA1 rs3077G/G is associated with HBeAg seroconversion and combined response rates at 6 months of the reply in $HBeAg^+$ CHB patients with IFN, suggesting HLA-DPA1 contributes to IFN-induced HBeAg seroconversion (*21*). Our previously published data demonstrates that polymorphisms in IPS-1 (rs2464 CC) are independently associated with response to PEG-IFN among Chinese $HBeAg^+$ CHB patients (*22*). The data from ourselves and others illustrates that different proteins regulated by SNPs contribute to HBV viral clearance and subsequent disease outcome *via* host antiviral immunity in response of interferon. However, it was still unclear whether SNP of IPS-1 polymorphisms influences IFN response in HBV infection.

2. Materials and Methods

2.1. *Cell lines and Culture*

HepG2 and HBV-replication-stable HepG2.2.15 cell lines were maintained in our laboratory, which were obtained from the Chinese Academy of Sciences (*23-25*). HEK293T cells, obtained from American Type Culture Collection, were used as a transfection positive control (*26*). HepG2, HepG2.2.15 and HEK293T cells were cultured in DMEM medium, supplemented with 10% fetal bovine serum at 37°C (Life Technologies, Carlsbad, CA), in a humidified atmosphere containing 5% CO_2.

2.2. *Site-directed Mutagenesis*

The sequences containing IPS-1 cDNA (FL18489) based on a human cDNA library were inserted into pCMV-SPORT6 plasmid, and then transfected into *E. coli* for amplification. The correct clone was confirmed with sequencing, and used as positive control. Primers were designed including the whole ORF sequences of IPS-1, joining enzyme loci Bgl II and Cal I at the both ends, respectively. In addition, KOZAK was also added as protection. The sequence of these two strains of primers were as follows: IPS-1seq-sense: ACCAGATCTGCCACCATGCCGT TTGCTGAAGACAAGACC; IPS-1seq-antisense: ATTGTCGACCTAGTGCAGACGCCGCCGGTA. IPS-1 cDNA fragment, inserted into pRRL-cPPT-PGK-eGFP-WPRE plasmid (Addgene#12252), was amplified for final identification.

Three point mutations, identified from each sequence of IPS-1 cDNA, were localized at the position of the SNPs of interest (Supplement Figure S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=40*). We used site-directed mutagenesis to obtain the wild-type vector of IPS-1 and other different expression vectors of SNP genotype. The primers for site-directed mutagenesis are listed in Supplement Table S1 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=40*). PCR was performed as follows: 94°C for 2 minutes, then 25 cycles at 94°C for 15 seconds, 56°C for 30 seconds, 68°C for 1 minute, and 68°C for 9 minutes.

2.3. *qRT-PCR*

HEK293T, HepG2 and HepG2.2.15 cells were transfected with human wild-type IPS-1 or different IPS-1 SNP genotypes (rs17857295, rs7262903 or rs7269320), respectively in triplicate plates. RNA was extracted from individual treated cells, using Trizol (Life Technologies, Carlsbad, CA). cDNA was generated from 1μg total RNA, using the RevertAid™ Reverse Transcriptase kit (Thermo Fisher Scientific, Waltham, MA). The forward and reverse primers for pgRNA are listed in Supplement

Table S2 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=40*). qRT-PCR was performed as follows: 95°C for 2 minutes, then 40 cycles at 95°C for 15 seconds, 60°C for 20 seconds, 72°C for 20 seconds, and 39 cycles at 72°C for 30 seconds.

2.4. *Western Blot*

Protein was extracted from these treated cells after lysing with RIPA buffer (Abcam, Cambridge, UK) for Western blot. Electrophoresis was performed after protein quantification using BCA kit (Abcam, Cambridge, UK). The blots were blocked and labeled with primary antibodies (rabbit anti-human IPS-1, Millipore, Darmstadt, Germany) overnight. Membranes were incubated with secondary antibody (goat anti-rabbit-HRP, Amersham Pharmacia Biotech, Saclay, France). The signal was developed and imaged using ImageQuant™ LAS 4000 (Fujifilm, Tokyo, Japan).

2.5. *Statistical analysis*

All data were expressed as the mean ± standard deviation (SD). Two-tailed Student's *t*-test and one-way ANOVA were used for data analysis. All statistical analyses were performed using the SPSS for Windows, Version 19.0 (SPSS Inc., Chicago, IL). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. *Construction of recombinant mammalian expressing vector with different IPS-1 SNP genotypes*

It was confirmed that constructions of recombinant mammalian expressing vector carried IPS-1 wild-type or other SNP genotypes (rs17857295, rs7262903 or rs7269320), by sequencing (Figure 1). Furthermore, to verify the ability to express IPS-1 properly, HEK293T cells (GFP gene contained in pRRL-cPPT-PGK-eGFP-WPRE plasmid) were transfected with the vectors, which carried wild-type or different IPS-1 SNPs. Thus GFP production in the cells indicates successful and stable transfection. IPS-1 mRNA from the wild-type or SNP mutated gene type transfected cells were significantly higher than that of mock transfected cells ($p < 0.05$) (Figure 2), confirming the vector plasmids were successfully generated with correct sequence.

3.2. *IPS-1 polymorphisms in HBV infection*

Subsequently, to determine the relationship between SNPs and IPS-1 expression during HBV infection in hepatocytes, HepG2 or HepG2.2.15 cells were transfected with the vectors containing IPS-1 SNPs or wild-type genotypes, respectively. IPS-1 gene

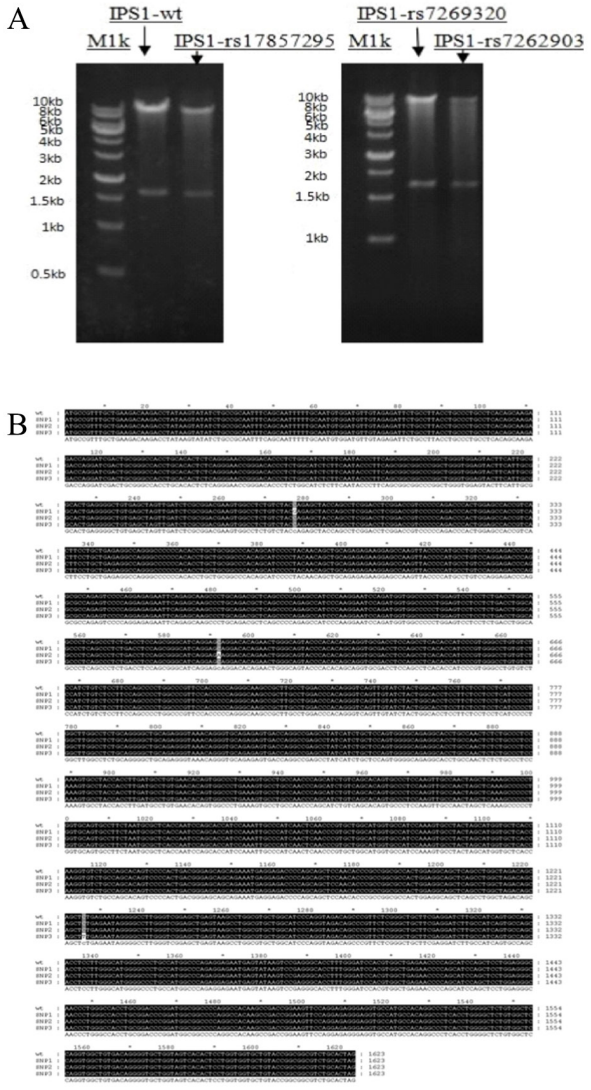

Figure 1. Construction of recombinant mammalian expressing vector with different IPS-1 SNP genotypes. (A). Incision enzyme digestion of different IPS-1 genotype plasmids; **(B).** Sequencing of different IPS-1 genotype plasmids (wt: IPS-1 wildtype, SNP1: rs17857295, SNP2: rs7269320, SNP3: rs7262903).

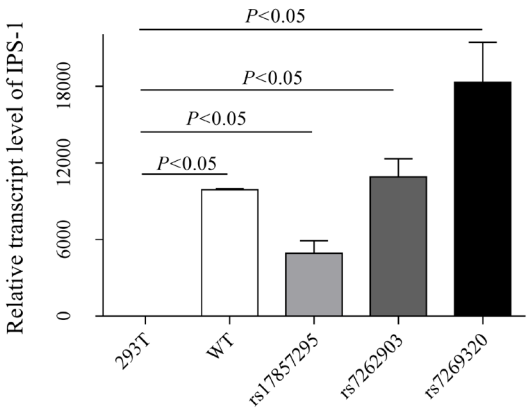

Figure 2. The expression of IPS-1 mRNA in different IPS-1 SNP genotype plasmids transfected 293T cells.

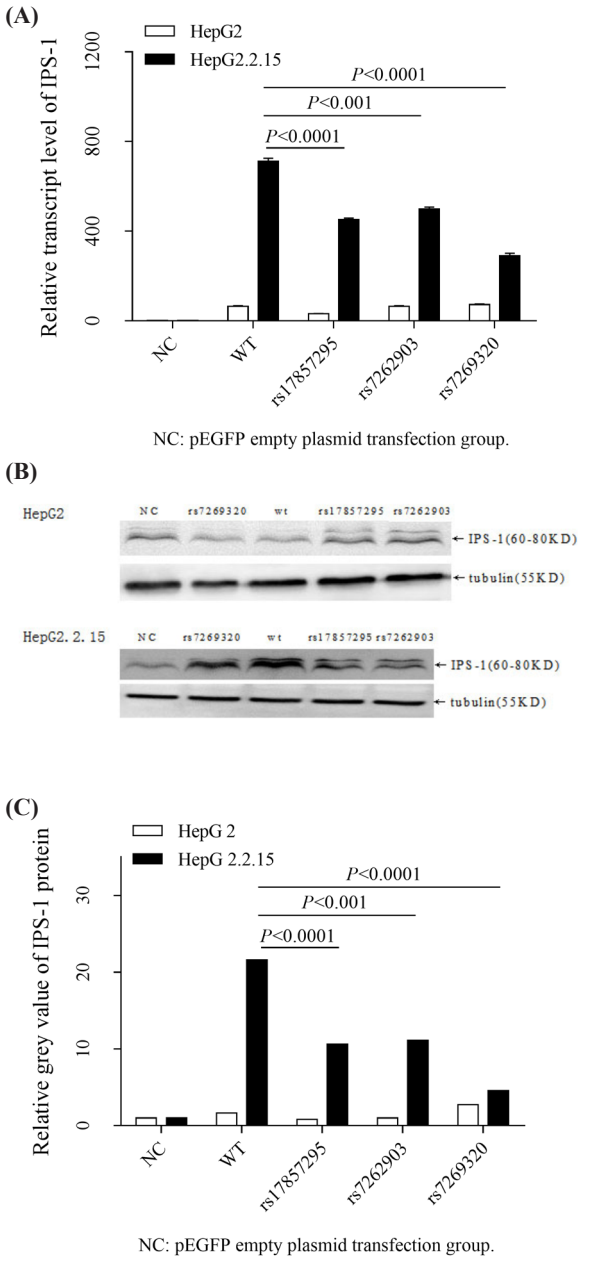

Figure 3. The expression of IPS-1 in different IPS-1 genotype plasmids transfected HepG2 or HepG2.2.15 groups. (A). The expression of IPS-1 mRNA in different IPS-1 genotype plasmids transfected HepG2 or HepG2.2.15 groups. **(B).** Western Blot detect different IPS-1 genotype plasmids transfected HepG2 or HepG2.2.15 groups. **(C).** The analysis of grey value among different IPS-1 genotype plasmids transfected HepG2 or HepG2.2.15 groups.

was significantly higher in the cells transfected with these three different IPS-1 SNPs or wild-type than the mock transfected group ($p < 0.05$). IPS-1 mRNA level in HepG2.2.15 cells transfected with wild-type, rs17857295, rs7262903, rs7269320 was 11, 14, 8 or 4 fold higher than HepG2 cells ($p < 0.05$). In contrast, IPS-1 mRNA level was 37%, 31%, 40% lower in the HepG2.2.15 cells transfected with IPS-1 rs17857295, rs7262903 or rs7269320 ($p < 0.05$) SNP genotypes than wild genotype transfection group (Figure 3A).

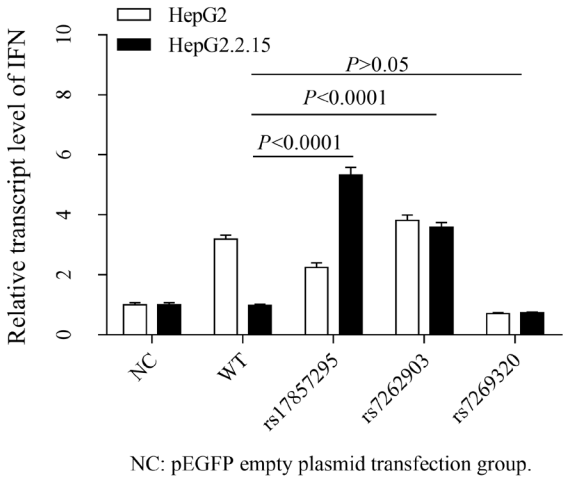

Figure 4. The expression of IFN-β in different IPS-1 genotype plasmids transfected HepG2 or HepG2.2.15 groups.

To confirm if the modification of IPS-1 was consistent between mRNA and protein, Western blot was conducted, demonstrating that IPS-1 was reduced by 49%, 52%, or 21% in the HepG2.2.15 cells transfected with rs17857295, rs7262903 or rs7269320 than that of wild-type transfection ($p < 0.05$) (Figure 3B and 3C). Interestingly, IPS-1 protein was 13, 13, 11, or 2 fold higher in HepG2.2.15 cells than in HepG2 cells with different transfection, *i.e.* wild-type, rs17857295, rs7262903, rs7269320 (Figure 3C).

3.3. *IPS-1 SNP polymorphisms regulate interferon response in HBV infection*

To determine the role of IPS-1 SNP polymorphisms in regulating interferon response in HBV infection, IFN-β mRNA level was detected after the vectors containing these three different IPS-1 SNPs or wild-type genotypes transfected into HepG2 or HepG2.2.15 cells. In HepG2.2.15 cells, IFN-β expression in IPS-1 wild-type vector transfected group had no obvious change compared to pEGFP empty control group. IFN-β mRNA level was 5.4 or 3.7 fold higher in the transfected HepG2.2.15 cells with IPS-1 rs17857295 or rs7262903 SNP genotypes than wild genotype transfected group ($p < 0.0001$). In IPS-1 rs7269320 SNP genotypes transfected groups, IFN-β expression had no obvious change compared to wild-type group. In addition, in IPS-1 rs17857295 genotype vectors transfected groups, the IFN-β mRNA level in HepG2.2.15 cells was > 2 fold higher than that in HepG2 cells ($p < 0.001$) (Figure 4).

4. Discussion

Following transfection of rs17857295 or rs7262903 in the HBV carried hepatocytes, IPS-1 was suppressed, but IFN-β was up-regulated compared to that with

wild-type transfection. IPS-1, but not IFN-β, was suppressed in the HBV carried hepatocytes transfected with rs7269320 SNP compared to that with wild-type transfection. IFN-β was higher in rs17857295 transfected HBV carried hepatocytes than in non-HBV carried hepatocytes.

IPS-1 plays a critical role in the RIG-I signaling pathway, leading to IFN-β activation in cells following viral stimulation (*9-11*). However, viruses may still circumvent IFN initiated host response *via* an unknown mechanism in susceptible individuals (*14,27*), resulting in chronic viral infection. Lin *et al*. (*28*) demonstrated that HCV protease NS3-4A inhibits IFN-β production *via* degrading IPS-1 protein. However, the effect of IPS-1 SNP polymorphism during HBV infection is still unclear. Among 29314bp DNA sequences within IPS-1 gene, 229 SNPs loci have been discovered. Considering the effects on the protein structure and function, we focused on the missense coding SNP (cSNP) sites within the IPS-1 gene. Three out of 12 missense cSNP sites show a mutation frequency which is > 1%, including rs17857295 in exon 3 (446 g>C, Q93E), rs7262903 in exon 5 (761 a>C, Q198K) and rs7269320 in exon 7 (1395 t>C, S409F). The encoding amino acids of rs17857295 loci is in the connection position between CARD structure domain and a proline rich area (Pro-rich), suggesting this SNP is closely related to the IPS-1function (*29*).

In the current study, IPS-1 was significantly higher in the wild type or SNPs transfected HBV carried hepatocytes (HepG2.2.15 cells) than that in non-HBV carried hepatocytes (HepG2 cells), suggesting that IPS-1 production is responding to HBV infection in the hepatocytes. Our data is in line with our previously published data in macrophages, IPS-1 in macrophages from CHB patients is significantly higher than that of good health controls following vesicular stomatitis virus stimulation (*26*).

Furthermore, IPS-1 was suppressed in any of the SNPs transfected (rs17857295, rs7262903 or rs7269320) HBV carried hepatocytes compared to that transfected with wild type. In contrast, IFN-β was substantially higher in the rs17857295 or rs7262903 SNP transfection HBV carried hepatocytes than that of the wild type transfected group. This is consistent with our previous finding that IPS-1 in macrophages from CHB patients is significantly higher than that of good health controls or acute hepatitis B, following vesicular stomatitis virus stimulation, inversely correlated with IFN-β production (*26*). Our current finding suggests that increased IPS-1 production from the virus stimulation in HBV carried hepatocytes is due to compensation for compromised IPS-1 function, and possibly due to interference from HBV infection. This is supported by the finding that HBx protein of HBV interferes the ubiquitination of IPS-1 to restrain the RIG-I pathway of innate immunity (*30*). However the underlying mechanisms leading to compromised IPS-1 function still remain to be explored.

Such findings invite speculation that the viral infection signals are delivered more effectively in rs17857295 and/or rs7262903 SNP transfected HBV viral carried hepatocytes compared with wild-type transfected ones, contributing to IFN-β mediated anti-viral immunity. In addition, IFN-β from rs17857295 transfected HBV carried hepatocytes was significantly higher than from non-HBV carried hepatocytes. No significantly different IFN-β production was detected between HBV carried and non-HBV carried hepatocytes following wild-type transfection, suggesting IPS-1 rs17857295 SNP may play an important role in host resistance to HBV challenge. Moreover, there was no significantly different IFN-β between wild type and rs7269320 SNP transfection, suggesting rs7269320 mutation may not be a critical factor or may be compensated by other factors in the viral host. The precise underlying mechanism will be determined in the future.

There are some limitations in our current study: First, the current study was mainly focused on basic virology. Thus future experiments may focus more on clinical research. Second, more downstream pathways of IPS-1 in RIG-I will be explored in the future. Third, in our current experiment there was correlated up-regulation of IPS-1 and IFN-β, but the viral load wasn't determined at the same time. However it is well known that IFN-β is responsible for viral clearance. Thus it invites speculation that IPS-1 rs17857295 or rs7262903 SNP genotype might contribute to viral clearance. The precise correlation between IPS-1 and viral clearance will be determined in our future study.

In conclusion, rs17957295 and rs7262903 IPS-1 SNP genotypes may contribute to viral clearance, especially rs17857295 SNP genotypes, in comparison with the wild-type or rs7269320 SNP genotypes in HBV infected patients.

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References

1. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH, Group R-HS. Risk of hepatocellular

carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006; 295:65-73.
2. Sarin SK, Kumar M, Lau GK, *et al*. Asian-Pacific clinical practice guidelines on the management of hepatitis B: A 2015 update. Hepatol Int. 2016; 10:1-98.
3. Morikawa K, Shimazaki T, Takeda R, Izumi T, Umumura M, Sakamoto N. Hepatitis B: Progress in understanding chronicity, the innate immune response, and cccDNA protection. Ann Transl Med. 2016; 4:337.
4. Collins FS, Patrinos A, Jordan E, Chakravarti A, Gesteland R, Walters L. New goals for the U.S. Human Genome Project: 1998-2003. Science. 1998; 282:682-689.
5. Moudi B, Heidari Z, Mahmoudzadeh-Sagheb H. Impact of host gene polymorphisms on susceptibility to chronic hepatitis B virus infection. Infect Genet Evol. 2016; 44:94-105.
6. Patel N, White SJ, Thompson RF, Bingham R, Weiss EU, Maskell DP, Zlotnick A, Dykeman E, Tuma R, Twarock R, Ranson NA, Stockley PG. HBV RNA pre-genome encodes specific motifs that mediate interactions with the viral core protein that promote nucleocapsid assembly. Nat Microbiol. 2017; 2:17098.
7. Beachboard DC, Horner SM. Innate immune evasion strategies of DNA and RNA viruses. Curr Opin Microbiol. 2016; 32:113-119.
8. Kell AM, Gale M, Jr. RIG-I in RNA virus recognition. Virology. 2015; 479-480:110-121.
9. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol. 2005; 6:981-988.
10. Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, Tschopp J. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 2005; 437:1167-1172.
11. Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell. 2005; 122:669-682.
12. Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, Shu HB. VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol Cell. 2005; 19:727-740.
13. Yang DR, Zhu HZ. Hepatitis C virus and antiviral innate immunity: Who wins at tug-of-war? World J Gastroenterol. 2015; 21:3786-3800.
14. Lu HL, Liao F. Melanoma differentiation-associated gene 5 senses hepatitis B virus and activates innate immune signaling to suppress virus replication. J Immunol. 2013; 191:3264-3276.
15. Thursz M. Genetic susceptibility in chronic viral hepatitis. Antiviral Res. 2001; 52:113-116.
16. Akcay IM, Katrinli S, Ozdil K, Doganay GD, Doganay L. Host genetic factors affecting hepatitis B infection outcomes: Insights from genome-wide association studies. World J Gastroenterol. 2018; 24:3347-3360.
17. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009; 461:399-401.
18. Kim SU, Song KJ, Chang HY, Shin EC, Park JY, Kim DY, Han KH, Chon CY, Ahn SH. Association between IL28B polymorphisms and spontaneous clearance of hepatitis B virus infection. PloS One. 2013; 8:e69166.
19. Sonneveld MJ, Wong VW, Woltman AM, Wong GL, Cakaloglu Y, Zeuzem S, Buster EH, Uitterlinden AG, Hansen BE, Chan HL, Janssen HL. Polymorphisms near IL28B and serologic response to peginterferon in HBeAg-positive patients with chronic hepatitis B. Gastroenterology. 2012; 142:513-520 e511.
20. Martin MP, Qi Y, Goedert JJ, Hussain SK, Kirk GD, Hoots WK, Buchbinder S, Carrington M, Thio CL. IL28B polymorphism does not determine outcomes of hepatitis B virus or HIV infection. J Infect Dis. 2010; 202:1749-1753.
21. Tseng TC, Yu ML, Liu CJ, Lin CL, Huang YW, Hsu CS, Liu CH, Kuo SF, Pan CJ, Yang SS, Su CW, Chen PJ, Chen DS, Kao JH. Effect of host and viral factors on hepatitis B e antigen-positive chronic hepatitis B patients receiving pegylated interferon-alpha-2a therapy. Antivir Ther. 2011; 16:629-637.
22. Wang H, Wu H, Bao S, Xiang X, Zhao G, Liu K, Li F, Xu Y, An B, Zhou H, Lu J, Xie Q. Association of IPS1 polymorphisms with peginterferon efficacy in chronic hepatitis B with HBeAg-positive in the Chinese population. Infect Genet Evol. 2015; 31:161-168.
23. Sells MA, Chen ML, Acs G. Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. Proc Natl Acad Sci U S A. 1987; 84:1005-1009.
24. Dong C, Qu L, Wang H, Wei L, Dong Y, Xiong S. Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. Antiviral Res. 2015; 118:110-117.
25. Wang H, Liu K, Fang BAM, Wu H, Li F, Xiang X, Tang W, Zhao G, Lin L, Bao S, Xie Q. Identification of acetyltransferase genes (HAT1 and KAT8) regulating HBV replication by RNAi screening. Cell Biosci. 2015; 5:66.
26. Zhao G, An B, Zhou H, Wang H, Xu Y, Xiang X, Dong Z, An F, Yu D, Wang W, Bao S, Xie Q. Impairment of the retinoic acid-inducible gene-I-IFN-beta signaling pathway in chronic hepatitis B virus infection. Int J Mol Med. 2012; 30:1498-1504.
27. Randall RE, Goodbourn S. Interferons and viruses: An interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol. 2008; 89:1-47.
28. Lin R, Lacoste J, Nakhaei P, Sun Q, Yang L, Paz S, Wilkinson P, Julkunen I, Vitour D, Meurs E, Hiscott J. Dissociation of a MAVS/IPS-1/VISA/Cardif-IKKepsilon molecular complex from the mitochondrial outer membrane by hepatitis C virus NS3-4A proteolytic cleavage. J Virol. 2006; 80:6072-6083.
29. Potter JA, Randall RE, Taylor GL. Crystal structure of human IPS-1/MAVS/VISA/Cardif caspase activation recruitment domain. BMC Struct Biol. 2008; 8:11.
30. Kumar M, Jung SY, Hodgson AJ, Madden CR, Qin J, Slagle BL. Hepatitis B virus regulatory HBx protein binds to adaptor protein IPS-1 and inhibits the activation of beta interferon. J Virol. 2011; 85:987-995.

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