**Gender disparity in chronic hepatitis B: Mechanisms of sex hormones**

Sheng-Han Wang,* Pei-Jer Chen*,†,‡,§ and Shiou-Hwei Yeh*,†

*Department of Microbiology and †NTU Center for Genomic Medicine and ‡Graduate Institute of Clinical Medicine and §Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University, College of Medicine, Taipei, Taiwan

**Abstract**

Hepatitis B virus (HBV) is a common human pathogen transmitted worldwide, and its chronic infection is a well-known risk factor for hepatocellular carcinoma (HCC). The sex disparity of HBV-related liver diseases has been noticed for a long time, which could be attributed to sex hormone effects, other than gender behaviors or environmental impact. This difference is experimentally confirmed in HBV transgenic mice, as well as in immuno-competent mice receiving hydrodynamic delivery of HBV. Androgen and estrogen pathways were identified to play opposite regulations of HBV transcription by targeting viral enhancer I at molecular level. In addition to the direct effects on HBV life cycle, sex hormones may be also involved in the immune response to HBV infection and the progression of associated liver diseases, although the detailed mechanisms are still unclear. Besides, several unaddressed issues such as HBV entry, microRNA profiles, viral integration, and adaptability in which androgen and estrogen axes might be involved are warranted to be delineated. The comprehensive understanding of the sex disparity in HBV virology and pathogenesis will be helpful to provide newly biomarkers for clinical diagnosis and develop novel drugs to manage HBV-related HCC patients.

**Introduction**

**HBV epidemiology.** Hepatitis B virus (HBV) is one of the most prevalent human pathogens worldwide, which chronically infects around 250 millions people and is considered to be a global health issue for a long time. HBV can be vertically transmitted from mother to infant at perinatal stage, which is the main way in endemic area such as Eastern Asia. In contrast, it is horizontally spread by parenteral exposure to infected blood or fluid to people in western countries with low prevalence rate. Individuals with chronic HBV infection are associated with hepatic inflammation and bear a high risk for liver cirrhosis and hepatocellular carcinoma (HCC), accounting for approximately 30% and 50% of total cases, respectively. To control HBV infection, the universal vaccination program had effectively reduced the hepatitis B surface antigen (HBsAg) carrier rate and to the decline of HCC incidence in newborns and children. However, adult carriers with chronic hepatitis B (CHB) are still under the risk of HCC development and need active antiviral therapies.

**HBV virology.** HBV is an enveloped virus containing partial double-stranded DNA as the genetic material, which belongs to one of the *Hepadnaviridae* family members. Hepatotropic infection of HBV is recently shown to be mediated by sodium taurocholate cotransporting polypeptide (NTCP), which is a membrane transporter responsible for hepatic uptake of bile salts. The N-terminus of the HBV large envelope protein was believed to interact with NTCP for cell attachment and viral entry. After infection of hepatocyte, the viral nucleocapsid is assumed to be transported to the nuclear pore where the HBV genome is delivered into the nucleus and repaired to be a covalently closed circular DNA (cccDNA) form. It can function as the template for the synthesis of four viral transcripts, of which the longest 3.5-kb pregenomic (pg) RNA encodes the core and polymerase proteins responsible for nucleocapsid formation and viral replication, respectively. Meanwhile, the pgRNA will be also served as the replication template for reverse transcription after encapsidation into core protein-derived nucleocapsid. The 2.4 and 2.1-kb viral messenger RNAs (mRNAs) are responsible for the expression of large, middle, and small surface proteins for constructing the viral envelope. These surfaces proteins are glycosylated in endothelium reticulum and assembled into an envelope for combining with nucleocapsid to a mature infectious virion. The shortest 0.7-kb viral transcript encodes a nonstructural HBV X protein (HBx) with versatile effects on both virus and host. The transcription of these viral mRNAs is controlled and relied on four distinct promoters and two master modulators, enhancer I and II. All these transcripts are coterminal through the recognition of a common polyadenylation signal.

In HBV life cycle, the transcription of pgRNA and other viral mRNAs in host cells is the key step for viral protein expression...
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and replication by reverse transcription. Because HBV replicates in a such error-prone manner, currently there are 10 genotypes designating from A to J reported in different geographic areas, whose genomic sequences are defined to be divergent over 8%. The variation of HBV genotypes has been linked to distinct pathological progression, and treatment outcomes and drug resistance.

Sex disparity of HBV infection in human and animal models

Clinical outcome of sex disparity in chronic HBV infection. HBV-related HCC occurs much more often in men than in women, with approximately 5–7:1 ratio. This epidemiological observation makes the male gender as an important risk factor for HBV-induced hepatocarcinogenesis. Of note is that this ratio of sex difference on the pathological outcome in HBV-infected patients are also reflected by disease progression, from 1.2 in asymptomatic carriers to 6.3 in CHB stage and finally 9.8 in HCC. The chronic HBV carrier prevalence was also observed to be higher in males than in females (10.7% vs 4.4%) with vaccination at birth and follow-up for over 18 years. Most importantly, in a large cohort case-control study from Taiwan, the elevated baseline of serum HBV titer was revealed to be significantly associated with male sex in chronic HBV patients, after matching and adjusting of other confounding factors. Since active HBV replication and higher serum viral loads in chronic HBV patients were clearly associated with increased risk of HCC development, these studies thus suggested the gender effect on HBV induced HCC started during the relative early stage of chronic hepatitis B, by influencing HBV titers and antigens through either modulating host immune responses indirectly or regulating viral gene expression directly.

Observation of sex disparity in HBV transgenic animal models. In the past few decades, studying HBV-induced hepatitis and HCC was difficult to be addressed in cell culture system, and it was usually delineated in an in vivo inbred mouse model. It is the first choice rather than other natural infection models due to the ease of laboratory manipulation, the expense of investigation budget and the debate of moral concern. However, mice are not natural host for HBV and viral entry of mouse hepatocytes is not yet possible (the human NTCP may be proved as a useful entry molecule in the future). To overcome this difficulty, various lineages of transgenic mice harboring HBV DNA genome by embryonic delivery were established by many groups. Notably, the sex disparity of serum viral loads and antigens which had been observed in clinical patients were also evident and reported in these HBV transgenic mouse models. Farza et al. established HBV transgenic mice containing an HBV genome except for the core gene, and found that serum HBsAg level was five to 10-fold higher in male mice than in female mice, which occurred in its viral transcript amounts and noticed after puberty. Intriguingly, the male preference of HBV S gene expression was compromised by testis castration but rescued by supplementation of androgen. The published results from two independent lines of HBV transgenic mice built by DeLoia et al. supported the role of androgen in maintaining higher serum HBsAg level in adult male mice. While the transgenic mice raised by Araki et al. displayed no sex difference in the titer of S and E antigens in animals at 7 weeks old, but it was not continuously followed up until older adult stage. The upregulating effect of androgen on serum HBsAg level in transgenic male mice was further shown to be mediated by androgen receptor (AR), because the HBsAg level was no more elevated in mice carrying mutant Tfmr allele of AR which lost 90% of protein activity, thus suggesting the regulatory effect of androgen/AR axis on HBV gene expression.

In 1995, the HBV transgenic mice generated by Guidotti et al. with the terminally redundant over-length 1.3-fold HBV DNA construct, showed comparable levels of serum viral particles and hepatic HBV DNA to those found in chronically infected patients. Although the sex difference of serum HBsAg level described above was not specifically mentioned in this original report, other groups applying this transgenic line for independent studies as well as the author’s follow-up investigation had clearly characterized the higher levels of HBV titers and viral antigens in male mice than in females. Consistent with this phenotype, and Chen et al. also provided evidences in recent years for the sex disparity of viral loads and antigens in the individually developed HBV transgenic mouse. Especially, in our transgenic mouse model, depletion of serum androgen in male mice by castration resulted in the 3.5-fold decrease of HBV titers. In contrast, deprivation of serum estrogen in female mice by ovariectomy led to the 3.5-fold increase of viral loads, while supplementation of estrogen in male mice repressed serum HBV DNA levels with an approximate 60% reduction. This highlighted the opposite regulatory effects of both sex hormones on HBV replication in vivo. In last year, the male preference of viral gene expression and replication was also demonstrated in the mice with the transgene of woodchuck hepatitis virus genome, a member of Hepadnaviridae family closely related to HBV. The results of sex disparity in HBV titers and/or antigens in many transgenic mice from different studies were summarized in Table 1.

The different lines of HBV transgenic mice produced by delivering the transgene of viral genome into embryos all exhibited adaptive immunity tolerance for HBV, except for the cases receiving adoptive transfer of HBV antigen-recognizing specific cytotoxic T cells. Since the immune system of these transgenic mice recognizes the virus as self-antigens, it thus substantially pointed out the direct effects elicited by androgen and estrogen sex hormones on HBV gene expression and replication, in addition to modulating HBV-specific immune responses in liver tissues.

Mechanisms of sex hormones on HBV life cycle

Sex hormones and liver. Androgen and estrogen are major sex hormones in men and women, which are mainly produced by testes and ovaries in adult stages, respectively. Both are originated from cholesterol as the initial 27-carbon backbone substrate and biosynthesized via a cascade catalysis by oxidative/reductive P450 enzymes. Then these gonad-derived sex hormones are released into systemic circulation to exert their biological functions in responsive tissues. Because sex hormones are small lipophilic molecules, they are easily diffused across the plasma membrane and directly bind to their cognate inactive nuclear receptors, AR or
androgen responsive elements (ARE) located in nucleotide 913–927 and 949–963 (with EcoRI site as nucleotide 1 in HBV genome) within HBV enhancer I, which was accessible to direct binding of AR and evolutionarily conserved in different HBV genome) within HBV enhancer I, which was accessible to direct binding of AR and evolutionarily conserved in different HBV genome. Disruption of these two ARE sites abrogated the binding of AR and evolutionarily conserved in different HBV genome. Disruption of these two ARE sites abrogated the binding of AR and evolutionarily conserved in different HBV genome.

Table 1 Sex disparity of HBV in transgenic mice

| Group | Publication | HBV transgene | Sex disparity |
|-------|-------------|----------------|--------------|
| Farza et al.19 | PNAS, 1987 | 0.86 x mer | Serum HBsAg is higher in male mice than in females |
| DeLoia et al.20 | J. Virol., 1989 | G7 line : 0.75 x mer nt 2400–3221 region deleted | In both lines, serum HBsAg is higher in male mice than in females |
| Araki et al.21 | PNAS, 1989 | 1.2 x mer | No sex difference in HBsAg and HBeAg levels shown at 7 weeks old but not mentioned thereafter |
| Guidotti et al.22 | J. Virol., 1995 | 1.3 x mer | Serum HBV titer, HBsAg, and HBeAg are higher in male mice than in females |
| Guidotti et al.23 | J. Virol., 1999 | From Enh I, via nt 3221/0 to Poly A site | Serum HBV titer is higher in male mice than in females |
| Julander et al.25 | Antiviral Res., 2003 | 1.3 x mer | Serum HBV titer and HBsAg are higher in male mice than in females |
| Peltekian et al.24 | J. Hepatol., 2005 | From Enh I, via nt 3221/0 to Poly A site | WHV gene expression and replication are higher in male mice than in females |
| Wang et al.27 | Hepatology, 2009 | 1.3 x mer | Serum HBV titer and HBsAg are higher in male mice than in females |
| Meng et al.30 | J. Virol., 2014 | 1.3 x mer (WHV) | Serum HBV titer and HBsAg are higher in male mice than in females |
| †All mice carried the HBV transgene except for the WHV transgenic mice applied by Meng et al. |
| ‡All mice carried the HBV transgene except for the ICR outbred strain applied by Chen et al. |

HbsAg, Hepatitis B surface antigen; HBeAg, Hepatitis B antigen; HBV, Hepatitis B virus; ICR, Institute of Cancer Research; WHV, Woodchuck Hepatitis Virus.

estrogen receptor (ER), within target cells. The ligand-activated sex hormone receptors classically fulfilled their bioactivities as transcriptional regulators by controlling downstream gene expression.31,32

Liver was recently considered as a sexually dimorphic organ, which expressed AR and estrogen receptor α (ERα, the dominant ER type in hepatocytes33), and thus was responsive to sex hormones for the disparity found in gene expression pattern, immune responses and xenobiotic metabolism between men and women.34,35 In our serial studies, HBx was shown to enhance hepatic AR activity in an androgen-dependent manner, which may amplify the sex difference occurred in HBV-infected male liver tissues.36,37 Besides, the transgenic animal studies described above suggested the liver in males is a more favorable environment for HBV gene expression and viral replication than in females, therefore implying the basic regulation of HBV virology by androgen and estrogen concomitantly in hepatocytes.

**HBV transcription regulated by sex hormone pathways.** To address this, first we demonstrated in 2009 that androgen-stimulated AR increased overall HBV transcription, including all of four viral mRNAs, which was validated in culture system and transgenic mice. Furthermore, we identified two androgen responsive elements (ARE) located in nucleotide 913–927 and 949–963 (with EcoRI site as nucleotide 1 in HBV genome) within HBV enhancer I, which was accessible to direct binding of AR and evolutionarily conserved in different HBV genotypes. Disruption of these two ARE sites abrogated the AR-mediated elevation for HBV mRNA levels, indicating these cis-regulatory elements were the major sites responsible for the transcriptional regulation of HBV by hepatic androgen/AR axis.27 This discovery characterized the ARE sites in HBV genome and thus proposed a positive feedback loop between AR and HBx for aberrantly activating and maintaining hepatic AR activity, leading to more active viral replication and higher oncogenic potential in male HBV patients. It provides a possible molecular explanation for the sex disparity of pathological outcome which starts from the stage of chronic hepatitis to HCC in HBV-infected population. Moreover, our results were confirmed by Tian et al. for the androgen/AR pathway in the upregulation of HBV mRNA levels in the hydrodynamic injection naïve mouse model.38 Similarly, the study from Wu et al. showed that hepatic AR promoted the HBV-induced hepatocarcinogenesis in HBV transgenic mice by enhancing HBV RNA transcription through direct binding to the ARE site we previously defined.29 These studies revealed that androgen stimulated HBV transcription through canonical AR pathway, leading to higher serum viral loads and antigens which raised HCC risk in chronically infected male patients.

In contrast to the increase of HBV mRNA synthesis by androgen/AR axis, the result from Almog et al. in 1992 showed that high dose (1 μM) of estrogen treatment reduced serum HBV E antigen (HBeAg) level in the HBV-stable HepG2.2.15 cell line which was examined by xenograft transplanted in immunocompromised male mice.40 Therefore, in addition to the androgen effect in males revealed by our group and others, the contribution of estrogen in females in jointly causing the sex disparity of HBV mRNA transcripts was conspicuous. Recently, we extended our concept about such regulation from female hormone pathway for HBV as well, and provided evidence to determine the exact role of estrogen in viral transcription. We found estrogen sustained the hepatic expression of its nuclear
receptor ERα, which reduced overall mRNA levels of HBV by suppressing the modulating activity of viral enhancer I. At the molecular level, we observed that ERα squelched the liver-enriched transcriptional factor hepatocyte nuclear factor 4α (HNF4α) and prevented it from binding to viral enhancer I, thus rendered the HBV transcription less active. Disruption of the HNF4α binding site which was highly conserved in different genotypes for HBV hepatotropism, abolished most of the ERα-induced repressive effect.29 Compared with androgen, our work in this study clearly defined the negative role of estrogen/ERα pathway in HBV transcription.

From these serial studies, HBV is considered to be a sex hormone responsive virus in essence, regardless of the sex disparity in host immune responses.41 The androgen-stimulated AR actively binds to the ARE sites within enhancer I, resulting in the enhancement of overall HBV mRNAs. In contrast, the mechanism of estrogen-sustained ERα squelched HNF4α and passively restricted its viral application for enhancer I, leading to the reduction of entire viral transcripts. The model displaying these molecular effects of both sex hormones on HBV enhancer I was illustrated in Figure 1. HBV enhancer I which is a master regulator responsible for viral transcription,42 is now refined to contain sequences targeted by both androgen and estrogen receptors.

Role of sex hormones in HBV-specific immune responses and disease progression

Sex hormones and HBV-specific immune response. Sex hormones play crucial roles in the regulation of immune system and the responses to exogenous pathogens. This sexually dimorphic nature is reflected by modulation of innate immune cells, maturation of adaptive lymphocytes, antigen presentation, and cytokine secretion. Androgen and estrogen are proved to influence systemic immunologic responses through classic hormone receptors expressed in the lineages of immune cells. Generally, in most cases of autoimmune diseases, there is a
higher incidence occurred in women than in men. For HBV infection, immune clearance of serum HBeAg and HBsAg was achieved in a higher percentage of female HBV patients than that of males. The seroconversion from HBeAg to anti-HBe and from HBsAg to anti-HBs were also observed more frequently in females than in male subjects. Besides, male gender was evident to be associated with poorer response for protection from HBV infection after vaccination in newborn stage. In HBV persistence mouse model, which was immunocompetent and received HBV genome by tail vein hydrodynamic injection, serum viral loads and antigens were shown to be lower in female mice than in males, consistent with the clinical chronic cases and HBV transgenic mice. Therefore, both humans and experimental animals displayed the sex disparity in HBV-related immune response, especially in immune tolerance and clearance phases.

The innate immunity is the first line to battle with HBV infection, which is sensed by toll-like receptors for recognizing HBV-associated molecular patterns, leading to activation of antiviral events, including intracellular defensive pathways and production of antiviral effectors such as interferons and pro-inflammatory cytokines. However, these detailed mechanisms is still not well defined due to the difficulty of clinical sampling and useful model systems. Besides, HBV seems to employ active strategies to evade innate immune surveillance after acute infection, as the lack of interferon response observed in infected experimental models.

So far, the impact of sex hormones on HBV evasion from host innate immunity in the early stage of viral infection remains largely unknown. In contrast, the HBV-specific CD8-positive T cell was well accepted as an important indicator of adaptive immunity for recognizing and removing HBV-infected hepatocytes entirely. Although the role of androgen and estrogen pathways in modulating T-cell responses to hepatic HBV infection is still unclear, their influences are proposed with some clues. For example, one of the identified single nucleotide polymorphisms (SNP) of ESR1 gene expressing ERα was associated with the susceptibility of persistent HBV infection in Chinese population, which may be exhibited by defective immune control of HBV and attributed to the unresponsiveness of the immune system to sex hormones. In addition, the androgen/AR axis in immune system was reported to exert immunosuppressive effects on development and activation of T cells. The percentage of T cells in the lymphocyte population was lower in males than in females, although the total count was similar in both sexes. In male mice, thymus enlargement and enhanced cellularity of thymocytes were observed after castration but reversed by androgen replacement, which could be due to the negative role of androgen on T-cell proliferation and differentiation. On the other hand, secreted HBeAg or even HBsAg was also proposed to be viral immunosuppressor to induce the exhaustion of helper T cells. However, it is reasonably to speculate that the sex disparity of host immune control for HBV infection may be partially achieved by directly regulating the expression of these viral antigens as described above. The detailed molecular mechanism for androgen and estrogen pathways in the immunopathogenesis in HBV infection warrants further investigations.

Sex hormones and HBV-related disease progression. The pathological progression of HBV-related liver disease displays the gender disparity as well as that occurs in HBV life cycle. From CHB, cirrhosis to HCC, male gender is an important risk factor for disease exacerbation as we mentioned above. Apart from regulating HBV transcription and modulating HBV-specific immune response, androgen and estrogen pathways were implicated to intensify or attenuate the HBV-induced liver pathology in chronic patients by interacting with host or viral factors.

In HBsAg-positive male HBV carriers, active androgen pathway was certified to be associated with an increased risk of HCC development, as indicated by both higher serum androgen levels and more activated AR gene alleles. Accordingly, in several years ago we provided evidences showing that HBx enhanced the transcriptional activity of AR in an androgen concentration-dependent manner, through aberrantly turning on the kinase switches such as c-Src and GSK3β for AR activation. Combined with the overall HBV transcription which can be stimulated by androgen, this host–virus interacting loop between AR and HBx is a positive feedback circuitry embedded in male HBV patients maliciously as we remarked above. With the hepatocarcinogenic potential of androgen/AR pathway well demonstrated in chemically induced HCC mouse models, our finding provided one of molecular mechanisms for explaining the gender disparity in HBV-related pathological outcomes. Dissecting the detailed cellular events evoked by aberrantly activated androgen pathway in men will be the next issue to be addressed for delaying or even treating male-specific cirrhosis and HCC beyond anti-HBV therapies. One of the examples as direct downstream genes controlled by hepatic AR was microRNA (miRNA) miR-216a. In our recent study, we discovered that the HBx-enhanced hepatic AR pathway upregulated the expression of miR-216a, resulting in a subsequent suppression of one of its targets gene, tumor suppressor in lung cancer-1 (TSLC1), as a novel mechanism for androgen axis in the early hepatocarcinogenesis of HBV-related male patients. Another case was cell cycle-related kinase (CCRK). It was overexpressed with AR in HBV-related HCC clinical specimens and proven to promote hepatocarcinogenesis through the upregulation of β-catenin/TCF signaling, which made it cooperated into the HBx-AR loop for regulating HBV transcription in a synergistic way.

In contrast to androgen pathway, the estrogen axis exerts protective effects and relieves the course of HBV-associated liver disease. Longer exposure to estrogen in female HBV carriers, such as the older age of menopause, taking oral contraceptives or post-menopausal hormone replacement therapy, was evident to be associated with a lower risk of HCC development. The specific SNP and haplotype of ESR1 were also believed to be susceptible to HBV-related cirrhosis and HCC in Chinese population, which might be probably caused by allele-specific expression of variant ERα forms with abnormal activity and dysregulated control by estrogen. In our previous study, wild-type ERα expression in liver tissues has been proven to significantly decreased in more than 70% of female HCC patients with HCC by a novel miR-18a-mediated targeting mechanism, suggesting the protective role of ERα for women. The ERα-mediated repression of interleukin-6 secretion from hepatic macrophage Kupffer cells was critical for alleviating the diethylnitrosamine-induced hepatocarcinogenic process in mouse model, indicating the role of estrogen pathway in shielding females from inflammation-induced liver injury. Furthermore, female estrogen was demonstrated to suppress hepatic fibrosis caused by repeated hepatic damage in a long time, as
evident in the enhanced fibrogenesis observed in female rats receiving ovarioectomy. For example, ERα activated by estrogen or its derivatives in persistently injured stage, also acts as an endogenous antioxidant to restrain the reactive oxygen species (ROS)-mediated cytotoxicity by inhibiting the activation of NF-kB, leading to the suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and decrement of ROS production. For HBV-infected hepatocytes, HBx was found to disturb the redox potential of mitochondrial transmembrane and enhance ROS generation, while it was suspected to be attenuated in women than in men due to the defensive effect of estrogen pathway. Therefore, in addition to passively repress HBV transcription by squelching HNF4α and subsequently lower viral titer and gene expression, the estrogen/ERα axis mitigates HBV-associated hepatic damage and delay the progression of disease as well, through cytokine modulation, ROS inhibition, or uncharacterized mechanisms. Whether the chelation of HNF4α by ERα at molecular level could be adopted to develop new management of liver injury warrants in-depth investigation.

Unaddressed fields of sex hormones in HBV virology and pathogenesis

Sex hormones and HBV viral entry. NTCP was recently identified as a major receptor for infectious entry of HBV. Its expression is well-correlated with the susceptibility of HBV infection. NTCP belongs to a kind of membrane transporter residing in the basolateral region of hepatocytes and is responsible for the hepatic uptake of mostly conjugated bile salts. Interestingly, female estrogen is well known to be associated with reversible intrahepatic cholestasis, which occurs in susceptible women with elevated serum estrogen levels such as pregnancy, administration of oral contraceptives, or hormone replacement therapy in postmenopausal stages. This estrogen-related abnormality is mediated by ERα and results in the transcriptional downregulation of NTCP gene. Accordingly, the sodium-dependent uptake of taurocholate by NTCP was confirmed to be indeed twofold lower in female rats than in males. The protein amount of NTCP expressed on the membrane of hepatocytes seems to exhibit the sex disparity, as observed in HBV-related diseases. Based on these previous studies, restraining hepatic expression of NTCP in females may be one possible mechanism for the estrogen/ERα axis as an anti-HBV guardian to restrict viral infection or spread in liver tissues, which is worth to be defined after establishing in vitro infection system.

Sex hormones and HBV-targeted microRNAs. MicroRNAs are small non-coding RNAs with approximately 22 nucleotides in length that have been regarded as key players in regulating gene expression post-transcriptionally by RNA interference pathway. They are evolutionarily conserved in eukaryotes from yeast to mammals, influencing various biological processes, including the control of host immunity and the responses to viral infection. The HBV transcriptional level was shown to be regulated by cellular miRNAs. It would be achieved by directly binding to HBV transcripts at post-transcriptional level, or by indirectly targeting to host transcriptional factors required for HBV gene expression. Notably, in our serial studies, we identified an ERα-targeted miRNA, miR-18a, which was significantly elevated in HBV-related female HCC cases due to its enhanced biogenesis aided by elevated/mutant p53. This sex-specific overexpression of miR-18a might reduce hepatic ERα protein amount, leading to increased HBV transcription through regulating the interaction effect of ERα and HNF4α on enhancer I in female HBV patients, as we discussed above. In contrast, so far none of cellular miRNA has displayed the regulatory effect on AR gene expression. Therefore, the profile of cellular miRNAs targeting androgen and estrogen pathways, other than HBV transcripts, is an extensive issue for discussing the sex hormone effects on HBV biology.

Sex hormones and HBV chromosomal integration. In HBV-infected hepatocytes, episomal form of HBV cccDNA frequently inserts itself into host chromosomes, which though is an unnecessary event in viral life cycle, but commonly believed to be one mechanism for hepatocarcinogenesis. Integrated HBV DNA was reported to be detectable in HBV-related HCC specimens with approximately 85–90%. Its existence was also found in non-tumor sections, and rare cases was even observed in acute hepatitis stages. The viral integration sites seem to be random throughout whole human genomes in chronic hepatitis, but the integration of some hot spots was found in specific genes such as hTERT and MLL4 in HCC. The influence of sex hormones for HBV chromosomal integration is still obscure and undetermined. However, the androgen pathway under genotoxic stress was verified to activate the enzymatic system for inducing chromosomal translocation in the proximal regions of AR-binding sites, which was an important driving force in the carcinogenic process of prostate. Because the ARE sites were also identified in HBV genome as we mentioned above, the integration probability of episomal HBV DNA reinforced by androgen pathway in men may be one of another mechanistic explanations for the sex disparity of HBV-related HCC development. Besides, it is an undiscovered field to determine the possible role of sex hormones in DNA damage and repair system responsible for chromosomal rearrangement and viral genome integration.

Sex hormones and HBV adaptability. Based on previous studies defining the sex disparity in HBV virology and pathogenesis, the host–virus interaction of sex hormones and HBV life cycle raises an evolutional issue for viral adaptability in men and women. The ARE sites and the binding site of HNF4α within HBV enhancer I both are highly conserved in different genotypes. However, via HNF4α, the estrogen/ERα axis in females targets this Achilles of HBV adapted in host cells to control viral transcription and replication. Undoubtedly, it is a beneficial response for host but unfavorable to virus, while some molecular events targeting estrogen pathway were noticed to compromise this defensive mechanism. For example, the exon 5-deleted mutant of ERα was demonstrated to be a dominant negative form which could cooperate with HBX to suppress the transcriptional activity of ERα. Compared with normal control, it was preferentially observed in HCC patients and proposed to be a negative predictor for prognostic survival. Besides, ESR1 was demonstrated to be one of the most frequently methylated genes in an HBV infection mouse model with chimeric human livers. These observations
were consistent with the association of \textit{ESR1} gene polymorphism with the susceptibility of HBV-related liver diseases, as we have referred above. Nevertheless, the particular events of these phenotypes obtained in HBV-infected hepatocytes are not fully understood and demanded for more studies.

**Conclusion and prospective**

The sex disparity of HBV-associated liver diseases has been recognized for long time, and it is conceptually ascribed to the opposite effects of sex hormone androgen and estrogen. Rather than life style and environmental impact, this difference is reproduced in experimental animal models and suggested to be motivated by sex hormones with genetic evidences. In HBV biology, androgen-activated AR actively binds to viral enhancer I and stimulates viral transcription comprehensively, while estrogen-sustained ERI passively squelches HNF4α from activating enhancer I and then subdues HBV transcription. The immune clearance of HBV antigens is faster to be achieved in women than in men, as well as the control and hold-up of progression in HBV-induced liver diseases. On the other hand, the underlying mechanism of HBV-specific immune responses regulated by androgen and estrogen is still uncertain and further studies are required in future. At the same time, the unknown roles of androgen and estrogen involved in HBV virology and pathogenesis, such as viral infection, microRNA influence, host chromosomal integration, and viral adaptability, warrant advanced investigations. After the discovery of NTCP as a major HBV receptor for viral entry, these unaddressed fields are believed to be more easily accessible by establishing \textit{in vitro} HBV infection culture system or transgenic mice. As a next step, sex hormone pathways in HBV-infected hepatocytes are potential targets, especially for blocking the stimulatory axis of androgen/AR which could interact with HBx. The liver-specific AR inhibitors, but not conventional anti-androgens with systemic adverse effects, are possibly to be developed for CHB patients. This will provide a novel concept for anti-HBV therapies and even new drugs to prevent or treat HBV-related male HCC. In contrast to suppressing hepatic AR pathway, the estrogen replacement therapy for sustaining ERI activity in liver tissues to inhibit HBV transcription is still an open and controversial question that should be carefully evaluated. Taken together, the understanding of HBV as a sex hormone responsive virus in essence helps explain the sex disparity of CHB related end-stage liver diseases, and may provide new insights of therapeutic development in future.

**Acknowledgement**

This work was supported by the grant from the Ministry of Science and Technology, Taiwan (MOST103-2321-B-002-025).

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