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Hepatitis B Virus, A Sex Hormone-Responsive Virus

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See “Estrogen receptor α represses transcription of HBV genes via interaction with hepatocyte nuclear factor 4 α ,” by Wang SH, Yeh SH, Lin WH, et al, on page 989.

Hepatitis B virus (HBV) can cause both acute and chronic infections, but only chronic infection leads to liver cirrhosis and hepatocellular carcinoma (HCC). A critical determinant for the resolution versus chronification of an acute infection is the age at transmission. In Western countries, HBV is spread horizontally during adulthood through sexual contact, shared needles, or blood transfusion, leading to a 5%–10% chronicity rate. In Eastern Asian countries such as China, Japan, and Taiwan, the most common mode of HBV transmission is around birth from chronically infected mothers positive for hepatitis B e antigen (HBeAg), and >90% of infections become chronic (Figure 1). The different modes of transmission can explain why the majority of the 300 million chronic carriers of HBV reside in East Asia. It is believed that the immature immune system of the infants together with the tolerogenic effects of HBeAg, a secreted version of the nucleocapsid protein, promotes the establishment of immune tolerance, while the high viral load found in HBeAg-positive blood or body fluids ensures infection. Clinical diseases of HBV infection do not occur in this early immune tolerance phase (first 2 decades or so), but rather at the later immune clearance phase, when repeated cycles of hepatocyte destruction, regeneration, and reinfection induce hepatitis and promote the risks for liver cirrhosis and HCC. In this endemic area of HBV infection, higher viral load, infection by genotype C instead of genotype B, and presence of specific mutations in the viral genome are associated with increased HCC risks.^{1–5}

Although women are primarily responsible for the transmission of chronic HBV infections in East Asia, HCC develops predominantly in chronically infected men instead, with a male-to-female ratio of 7:1. Although alcohol intake may partly explain the gender difference, studies from Taiwan suggest that higher blood testosterone concentrations and more active androgen receptor (AR) alleles in male HBV carriers are risk factors for HCC.^{6,7} Although liver is an organ of the digestive system, it does express AR and estrogen receptor (ER). AR translocates to the nucleus following ligand binding to activate target gene transcription. Five years ago, independent studies from Ou’s laboratory at University of Southern California and Chen’s team from the National Taiwan University demonstrated that 1 of the

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HBV gene products, the HBx protein, could interact with AR. Moreover, androgen-dependent transactivation activity of AR can be further enhanced by HBx.^{8,9} HBx probably exerts its effect by enhancing ligand-dependent AR binding to the promoter sequences of its target genes,⁸ which in turn is mediated by increased AR dimerization and activation of the transactivation domain through 2 protein kinases.¹⁰ In a mouse hepatocyte cell line, coexpression of HBx protein also moderately increased anchorage-independent colony formation by AR, suggesting its potential to augment AR-mediated hepatocarcinogenesis.⁹ Indeed, several mouse lines expressing the HBx protein alone displayed male predominance in HCC development. Certainly, data generated from HBx protein expressed alone should be treated with caution, especially if it is driven by a strong exogenous promoter. This is because HBx is expressed at very low levels from the full-length HBV genome. In fact, HBV can trigger HCC formation in the mouse model when combined with a chemical carcinogen, even when HBx is not expressed.¹¹

Because a high viral load is a strong risk factor for HCC,^{3,12} the Taiwanese group led by Yeh and Chen used transgenic mice and cell culture to experimentally test the gender effect on HBV replication. Interestingly, 3- to 4-month-old adult male mice showed higher serum titers of both viral DNA and hepatitis B surface antigen (HBsAg), the secreted viral envelope proteins, than female mice.¹³ Castration reduced both HBV DNA and HBsAg titers in male mice. Northern blot analysis revealed higher levels of all the major HBV transcripts in the liver of male than female mice, suggesting the immediate effect of the androgen pathway is on HBV RNA transcription. In the HepG2 human hepatoma cell line cotransfected with HBV DNA and AR expression construct, the androgen agonist R1881 augmented all the HBV transcripts. In this regard, transcription of the 3.5-, 2.4-, 2.1-, and 0.7-kb HBV RNAs is not only individually controlled by their respective promoters, but also collectively by the HBx protein and 2 enhancer elements, Enh I and Enh II. Experiments in HepG2 cells suggest that the effect of androgen pathway on HBV RNA transcription is independent of HBx protein. Using reporter constructs, the authors mapped the androgen-responsive element to nucleotides 899–1036 at the 5' end of Enh I, which physically interacts with ligand-stimulated AR. Further mutational analysis identified 2 androgen response elements within this region of Enh I (Figure 1).¹³

Very recently, the findings from the Taiwanese team were confirmed and extended by Ou's laboratory. They validated that male gender was associated with higher HBsAg titer as well as intrahepatic replicative HBV DNA and transcripts in adult (10-week-old) but not prepubescent (4-week-old) mice.¹⁴ Male gender was still associated with increased HBsAg secretion and HBV DNA replication when the HBV transgenome could not express HBx. Castration reduced HBV markers in male mice, which could be rescued by injection of R1881, the androgen agonist. Importantly, AR expression was much higher in adult than prepubescent mice, and short hairpin (sh) RNA-mediated knockdown of AR expression reduced HBV DNA replication, even more profoundly than castration, in these animals. Finally, mutating the 2 androgen response elements within Enh I reduced HBV genome replication, although castration further reduced viral replication.¹⁴ Overall, these findings establish that, in vivo, androgen-AR complex rather than androgen alone activates HBV RNA transcription through binding to the 2 response elements inside Enh I.

The male sex hormone, however, does not fully explain the gender disparity of liver cancer. For example, ovariectomy increased incidence of chemically induced HCC in female mice.¹⁵ When analyzing the impact of the age of menopause, number of pregnancies, ovariectomy, and use of oral contraceptives or postmenopausal hormonal replacement therapy on HCC development in women, Yu et al¹⁶ revealed a protective role of estrogen. In a previous issue of *Gastroenterology*, Yeh and Chen's group found that a microRNA, miR-18a, was significantly up-regulated in female HCC liver samples compared with control livers, and this microRNA blocked ER expression by binding to the 3' untranslated region of its mRNA.¹⁷ Therefore, HCC in females is significantly associated with diminished ER expression. Prompted by these findings, the Taiwanese group further examined effect of the estrogen pathway on HBV replication. As reported in the current issue of *Gastroenterology*, Wang et al¹⁸ found that ovariectomy in female HBV transgenic mice increased serum HBV DNA titer by > 3-fold, whereas implanting estrogen into male mice reduced HBV titer by 60%. These 2 lines of evidence establish that besides androgen, the estrogen axis also regulates HBV DNA replication.

The authors also found that estrogen up regulates hepatic expression of its receptor, ER α . To elucidate the mechanism whereby estrogen and/or its receptor suppresses HBV replication, additional experiments were performed in the HepG2 cell line. Transfection of human ER α cDNA diminished transcription of all the major HBV RNAs, irrespective of the expression status of HBx. Whereas AR requires androgen to augment HBV RNA transcription,¹³ ER α suppresses HBV RNA transcription independent of ligand (estrogen) association. Report assay revealed that ER α suppresses Enh I but not Enh II, and the responsive sequence was mapped to nucleotides 1004–1252. Within this region are binding sites for several transcription factors, and over-expression of HNF4 α but not HNF3 or STAT3 could partially overcome the suppressive effect of ER α on the enhancer activity. Indeed the hinge region of ER α could physically interact with HNF4 α , which diminished HNF4 α binding to Enh I. Mutating the HNF4 site in Enh I markedly diminished enhancer activity of the reporter construct and also impaired HBV RNA transcription from the full-length HBV genome. Importantly, the mutant reporter construct and full-length genome could no longer be suppressed by ER α .

This is an elegant study combining in vivo observation with in-depth molecular characterization. Although previous studies from the Taiwanese team and Ou's laboratory independently demonstrated ability of the male hormone pathway to regulate HBV gene expression, genome replication, and virion production at the transcriptional level, at least in the mouse model and a human liver cell line, the current report extends such regulation to the female hormone pathway as well. It is not surprising that both androgen and estrogen pathways target an enhancer element within the HBV genome, such that the ratios among different viral proteins, and between virions and subviral (HBsAg) particles, are not distorted between male and female mice. The relevance of such findings to natural HBV infection in humans remains to be confirmed. For example, is the viral load lower in female HBV carriers taking oral contraceptives than age-matched controls, or does ovariectomy increase HBV titer? HBV can infect immune-deficient mice repopulated with human hepatocytes.^{19–20} It will be interesting to determine whether, in this simplified system, castration or ovariectomy alters viral load. Interestingly, a study from China revealed

association of a single nucleotide polymorphism in the ER α gene with the development of chronic infection, although the impact of the polymorphism on ER α expression has not been established.²¹ From a mechanistic point of view, ER targets a major liver-specific transcription factor, rather than Enh I itself. It is not immediately clear why ER α does not diminish the activity of the core promoter (the promoter for the 3.5-kb HBV RNAs), which also contains an HNF4 site. At any rate, we can now call HBV a sex hormone-responsive virus.

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Figure 1.

Gender disparity in the causation and consequence of chronic HBV infection in East Asia. Women are responsible for majority of chronic HBV infection but are less likely to develop HCC. The sex hormones and their receptors can either directly affect hepatocyte proliferation/transformation, or modulate HBV replication/protein expression at the transcriptional level, thus indirectly altering HCC risk.