学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Studies on Innate Immune Activation by HBV Infection and Its Sensing Mechanism in Hepatocytes

（HBV 感染による肝細胞における自然免疫応答とその認識機構に関する研究）

Pattern recognition receptors (PRRs) recognize invading viruses mainly by sensing viral nucleic acid and trigger key antiviral immune responses in the innate immune system that act as a front line of host defense against viral infection. Hepatitis B virus (HBV) is a hepatotropic DNA virus that can cause both acute and chronic liver disease, and more than 240 million people worldwide are chronic HBV carriers that have a high risk for liver cirrhosis or hepatocellular carcinoma. Current approved therapies of chronic hepatitis B include administration of antivirally active interferon-α (IFN-α) and inhibitors of viral reverse transcription have severe side effects and a risk to develop of drug resistant mutations. The nucleic acid sensor(s) for HBV and the host innate immune response against HBV infection has not been identified. Therefore, understanding of the nature of innate immunity induced by HBV will aid to characterize the immunopathogenesis of HBV infection and to further develop novel innate immune-based antiviral approaches for HBV infection or optimize the current therapy regimens of HBV. Thus, this study tried to elucidate the innate immunity activation upon HBV infection by analyzing the induction of IFNs and cytokines in several HBV infection models, and also clarify the mechanism of the innate immunity activation by HBV through analyzing the role of several innate immune response associated molecules in HBV infection.

The results of this study demonstrated that type III but not type I IFNs are predominantly induced in human hepatocytes in response to HBV infection, through retinoic acid-inducible gene-I (RIG-I)-mediated sensing of the 5'-ε region of HBV pregenomic RNA (pgRNA), and the induced type III IFN exhibits potent physiological antiviral activities. In addition, RIG-I could also directly suppress HBV replication in an IFN signaling-independent manner that counteracts the interaction of HBV polymerase (P protein) with the 5'-ε region, which is essential for initiation of viral pgRNA encapsidation during HBV replication. Furthermore, liposome-mediated delivery and vector-based expression of this ε region-derived RNA (εRNA) in liver abolished the HBV replication in human

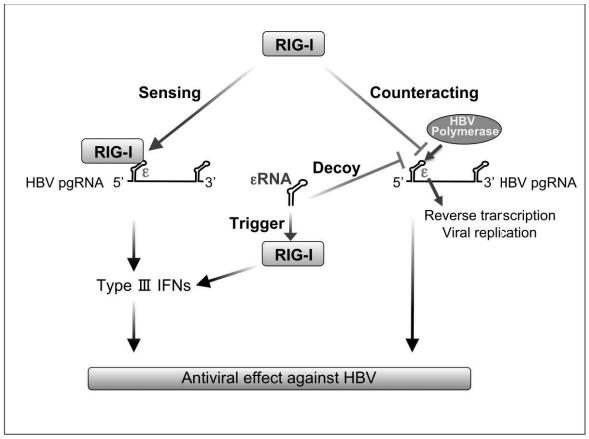

Figure 1. A schematic model showing the role of RIG-I and εRNA in the inhibition of HBV replication.

hepatocyte-chimeric mice.

The observations of this study reveal an innate recognition mechanism by which RIG-I dually functions as an HBV sensor activating innate signaling and to counteract viral polymerase in human hepatocytes (Figure 1). Moreover, this study also evaluate the therapeutic potential of the εRNA for the control of HBV infection, which may provide a better approach to the strategies for development of nucleic acid medicine, and offer an attractive clinical option for the therapy against not only HBV but also possibly other virus infections.

The structure of present thesis is as follows:

Chapter 1 is introduction. In this part, I reviewed the HBV, including the HBV history, structure, genomic organization, protein function, genotype, replication and experimental models for HBV research. Next, I summarized the innate immune responses, mainly described the PRRs, and also reviewed the innate immune responses after HBV infection. The introduction of therapeutic approaches for HBV are also given. Finally, the aims of this study is described in the last of this chapter.

In chapter 2, the description of the experimental details such as materials and methods are given.

In chapter 3, the results of this study are presented. At first, by using the *in vitro* and *in vivo* models that transfected or infected with HBV, I found that type III but not type I IFNs were predominantly induced in human hepatocytes in response to HBV infection. Next, I examined which nucleic acid sensor(s) is/are response for the induction of this type III IFNs by using siRNA-mediated gene knockdown in human hepatocytes, the study revealed that the RNA sensor RIG-I was critical for inducing type III IFNs expression after HBV infection. To further clarify how RIG-I recognizes HBV, this study next determined the ligand(s) of HBV that are responsible for the induction of type III IFNs, and indicated that the 5'-ε region of HBV pregenomic RNA (pgRNA) was critical for type III IFNs induction through the recognition by RIG-I. As the 5'- ε region of HBV pgRNA is critical for initiation of encapsidation and HBV polymerase binding for reverse transcription during HBV replication, this study further demonstrated that HBV could counteract the interaction of HBV polymerase with the 5'- ε region of pgRNA to directly suppress HBV replication in an IFN signaling-independent manner. And in consideration of the important role of ε region in both viral replication and RIG-I-mediated type III IFNs induction, the last of this study tried to evaluate the therapeutic potential of the εRNA for the control of HBV infection, and the results showed that vector-based expression of εRNA could suppress HBV replication in both cell culture model and human hepatocyte-chimeric mice model.

In chapter 4, the general discussion of the present thesis is given.