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News & Views

How the Key Finds its Door – Identification of HBV Receptor

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HBV is the predominant pathogen associated with hepatitis cases in China. Although the HBV replication mechanism has been extensively documented in recent years, the virus entry mechanism remains elusive; in particular, the HBV receptor has yet to be identified. Recently, a research team led by Dr. Wenhui Li finally identified Sodium taurocholate cotransporting polypeptide (NTCP) as the receptor for HBV infection of hepatocytes (Yan H, et al., 2012). This review highlights their research strategy, as well as the significance of the identification of the HBV receptor.

Hepatitis B is one of the most widely-spread and dangerous diseases and is caused by Hepatitis B virus (HBV) infection. Although the HBV replication mechanism has been extensively documented in recent years, the virus entry mechanism remains elusive. Early experiments indicate that the Pre-S1 of the HBV large surface protein appears to be associated with virus entry, as an N-terminal myristoylated peptide consisting of amino acids (aa) 2–48 of the pre-S1 domain can substantially block HBV infection of hepatocytes (Barrera A, et al., 2005; Engelke M, et al., 2006; Glebe D, et al., 2005; Gripon P, et al., 2005; Schulze A, et al., 2010). This evidence suggests a putative interaction partner of Pre-S1 on the surface of host hepatocytes may serve as an HBV receptor. Furthermore, several cellular proteins, including interleukin-6 (Neurath A R, et al., 1992), human squamous cell carcinoma antigen 1 (De Falco S, et al., 2001), and lipoprotein lipase (Deng Q, et al., 2007) were found to interact with Pre-S1 or facilitate HBV binding to the cell surface, whereas exogenous expression of these candidate receptors failed to confer susceptibility for HBV infection.

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Recently, a research team led by Dr. Wenhui Li at the National Institute of Biological Sciences, Beijing finally identified Sodium taurocholate cotransporting polypeptide (NTCP), a multiple transmembrane transporter predominantly expressed in the liver and involved in the circulation of bile acids, as the receptor for HBV infection of hepatocytes.

To setup a robust cell culture system to screen HBV receptor, the researchers isolated liver cells from treeshrew, a unique small animal that is susceptible to human HBV infection. Then they synthesized myristoylated peptides bearing the HBV Pre-S1 sequence with photo-reactive leucine incorporated as bait to bind to the surface of isolated treeshrew liver cells. The bait binds to its targets by protein-protein interaction, which is further enhanced by UV photo-reactive leucine-mediated covalent crosslinking. After tandem affinity purification (TAP), the protein complex containing the synthetic peptides and its interaction partners were isolated and submitted to mass spectrometry. By searching against a comprehensive treeshrew protein sequence database, they finally identified NTCP as the interaction partner of their synthetic peptides and which may serve as the HBV receptor. The researchers also provided evidence that showed exogenously expressed NTCP confers HBV susceptibility to Huh-7 and HepG2 cells, which are non-HBV-susceptible cells in their native state. To date, the authors have not provided further *in vivo* evidence that HBV can infect a transgenic animal bearing the NTCP receptor.

One of the keys to their success of identifying the HBV receptor was selecting the treeshrew as the animal model for HBV infection. Until now, only three species are known to be susceptible to human HBV infection, i.e. human, chimpanzee, and treeshrew. Since HBV receptor screen requires isolation of huge amounts of primary liver cells, it is almost impossible to get enough primary liver

cells from human or chimpanzee due to bioethics and cost concerns. In contrast, the treeshrew is a small mammal that is susceptible to human HBV but capable of providing sufficient primary liver cells at low cost. The only disadvantage of using treeshrew for screening HBV receptors is lack of transcriptome or proteome information. To overcome this obstacle, the research team led by Dr. Li established their unique transcriptome database of treeshrew by deep sequencing to ensure the success of identifying Pre-S1 peptide binding partner by Mass spectrometry.

Another essential strategy they employed is to combine a highly sensitive protein trap technique (photo-reactive leucine based UV crosslinking) with a highly stringent protein purification protocol (TAP). The HBV receptor is supposed to be either an extremely low level protein or low binding affinity protein. They synthesized ligand peptide (bait) with photo-reactive leucine that covalently crosslinks to the HBV receptor (prey), which ensures the bait-prey interaction is strong enough to capture as many receptors as possible. After they accumulated sufficient ligand-receptor complexes, a tandem affinity purification step was used to remove probably false protein complexes and ensure only bona fide ligand-receptor complexes were left for the mass spectrometry.

The discovery of the HBV receptor will definitely bring HBV research into a new era. As a research tool, it provides an ideal cell culture system to study HBV infection. In contrast to the commercially available HepaRG cell that supports HBV infection but needs prolonged and specialized cell culture procedures, the NTCP stably transfected HepG2 cells (HepG2-hNTCP) developed by Dr. Li provides an easy-to-use cell culture system susceptible for HBV infection with routine cell culture conditions. In fact, susceptibility of HepaRG cells to HBV infection is also due to its up-regulated NTCP receptor induced by exogenous dimethylsulfoxide as also demonstrated by Dr. Li.

Receptor degradation-recycling usually plays an essential role in regulating receptor functionality. The phenotype described in this research that NTCP is down-regulated rapidly after passage of primary liver cells raises the importance of exploring the NTCP degradation-recycling mechanism. Such insight into the NTCP degradation-recycling will not only help to develop a more robust HBV cell culture system (to suppress NTCP degradation), but also provide novel drug targets to suppress HBV infection (by promoting NTCP degradation).

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