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分担研究報告書（平成26年度）

Analyzing host factors regulating HBV as tool to develop HBV drugs and mouse model.

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研究要旨

There is no immunocompetent small animal model that is permissive to hepatitis B virus (HBV) infection. We are trying to identify the host factors that are required for the species restriction of HBV infection in human and chimpanzees, and use it to construct an immune-competent transgenic mouse that is permissive for HBV. Also we are trying to develop new anti-HBV drugs targeting the host factors required for HBV life cycle. The block of HBV infection in mouse cells is reported to be at the entry level, since all other parts of HBV life cycle can be efficiently recapitulated in mouse. Recently, sodium taurocholate transporter (NTCP) was discovered as a new entry receptor for HBV infection. However, no HBV infection was reported in mouse hepatocytes expressing human NTCP, suggesting either the lack of other host factor required for HBV infection, or the presence of a host inhibitory pathway in the mouse. We are screening for host factors affecting HBV life cycle and studying its effect on HBV infectivity on mouse hepatocytes, and the discovery of new anti-HBV drugs targeting these host factors.

A. 研究目的

The aim of this study is to understand the anti-HBV immune response and to utilize this knowledge for the development of novel and evidence-based therapeutic regimen for chronic HBV infection. To accomplish this, the primary aim of this study is to first establish an immunecomptent small animal model supporting HBV infection. The intermediate objective is to assess if an evidence-based and innovative therapy can be developed for chronic HBV-infected subjects with retrieved information. The final target is to provide a strategy and road map of immune therapy for HBV patients.

B. 研究方法

Host factors affecting HBV life cycle:

We work to identify host factors regulating HBV life cycle, and design new anti-HBV drugs targeting these factors.

1- Screening for human kinases regulating HBV replication: (started from 2013)

Using HepG2-AD38.7 cells in which HBV replication is inducible by tetracycline off system, and kinase siRNA (501 genes) library, we screened for those regulating HBV replication in the cell. According to the function of each kinase on HBV life cycle, we classified our results into kinases suppressing HBV, and kinases required for HBV life cycle.

Mechanistic Analysis of TSSK2 function on HBV replication.

We Used protein/protein interaction studies, overexpression and silencing, deletion mutants, RNA/protein interaction, to analyzed the mechanism by which TSSK2 suppresses HBV replication in the cell. As output for measuring the effect on HBV, we used southern blot, and real time PCR to measure HBV-DNA, we measured nuclease resistant core associated DNA, we used northern blot, and real time-RT-PCR for the detection of HBV-RNA, we used western blot for the detection of HBV proteins, and we constructed core, S1, S2, and X promoter luciferase reporter plasmids to assay the effect on transcription.

2- Screening for human helicases (133 genes), GPCR (380 genes), Nuclear receptors (52 genes), Cytokine receptors (116 genes).

Using HepG2-AD38.7 cells in which HBV

replication is inducible by tetracycline off system, and siRNA libraries, we screened for those regulating HBV replication in the cell. According to the function of each kinase on HBV life cycle, we classified our results into factors suppressing HBV, and factors required for HBV life cycle.

（倫理面への配慮）Ethical

All mice that will be used in this study will receive human care and permissions from institutional review board to conduct the study.

C. 研究結果

Host factors affecting HBV life cycle:

1- A) We screened 1182 host genes affecting HBV replication covering the following: 1- Kinases (501 genes), 2- Helicases (133 genes), 3- G-protein coupled receptors (380 genes), 4- Cytokine receptors (116 genes), 5- Nuclear receptors (52 genes).

B) We are currently analyzed by mechanistic analysis.

2- Using the human Kinase library, we identified a new pathway suppressing HBV replication.

A) We found that Testis Specific Serine Kinase 2 (TSSK2) expression was induced in liver cells in response to HBV infection.

B) TSSK2 kinase suppress HBV replication through its interaction with Superkiller Viralicidic Activity 2-Like **(**SKIV2L) helicase.

C) TSSK2 phosphorylates SKIV2L helicase, and this phosphorylation is important for the binding between SKIV2L and HBV-mRNA.

D) This is followed by SKIV2L mediated HBV-mRNA degradation through the RNA exosome.

D. 考察

This year in the HBV international meeting, it was reported that while HBV and HDV are using the same HBV surface antigen to attach and infect hepatocytes; HDV infection was possible in mouse hepatocytes expressing human NTCP (the newly identified HBV receptor) but not HBV. This suggest that the problem of permissiveness of mouse hepatocytes to HBV infection is not at the hepatocyte surface, but may be another host factor is required in the early steps of HBV infection, or an inhibitory pathway in the mouse suppressing HBV infection in the early stages. To identify the human host factors required for HBV life cycle and its effect on HBV infectivity when overexpressed in human NTCP expressing mouse hepatocytes, we used siRNA library screening.

Using the Kinase library screening, we discovered TSSK2 kinase to be induced by HBV replication in the cells. We also found that TSSK2 further phosphorylates SKIV2L helicase. SKIV2L helicase was known to identify and degrade invading viral RNA in the yeast. RNA degradation is carried through its interaction with the RNA exosome complex. No similar phenomenon was yet reported in human. We showed that phosphorylation of SKIV2L helicase in human by TSSK2 is important to bind with both HBV-mRNA and RNA exosome, and is required for the degradation of HBV-mRNA through the exosome complex.

We are recently planning to use this phenomenon to

1- To study its effect on HBV permissiveness of mouse hepatocytes

2- To develop a new anti-HBV drug targeting the degradation of HBV-mRN.

E. 結論

RNA exosome complex plays an important role regulating HBV-mRNA levels. This is mediated through the interaction with HBV-mRNA bound to SKIV2L helicase. SKIV2L helicase phosphorylation by TSSK2 kinase is required for this binding. We are aiming to use

this phenomenon to develop a new anti-HBV drug targeting the degradation of HBV-mRNA.

F. 健康危険情報

なしです

G. 研究発表

1. 論文発表

今年はなしです

2. 学会発表

1- Aly HH, Watashi K, Chayama K, Wakita T. Analyzing new host mechanism suppressing hepatitis B virus replication. 2014 TASL-Japan Hepatitis B Workshop (Second). Oral presentation, Taipei, Taiwan

2- Aly HH, Watashi K, Wakita T, Chayama K. The identification of a new interferon-independent host mechanism suppressing hepatitis B virus replication. 2014 International Meeting on Molecular Biology of Hepatitis B Viruses, Los Angeles (USA), Sep, 2014

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3- Aly HH, Watashi K, Chayama K, Wakita T. Host factors interacting with Hepatitis B virus life cycle. Egyptian-Japanese day, molecular biology of hepatitis viruses. Oral presentation, 2014, Cairo, Egypt.

4- Aly HH, Watashi K, Chayama K, Wakita T. The identification of a new host mechanism suppressing Hepatitis B virus replication. The 62nd meeting of the Japanese society of virology. Oral presentation, 2014, Yokohama, Japan.

5- Aly HH The identification of a new interferon independent anti-hepatitis B virus pathway. The second Japan-Italy hepatitis meeting. Oral presentation, 2014, Hiroshima, Japan.

6- Aly HH, the 11th JSH Single Topic Conference. SKIV2L helicase suppress HBV replication in interferon independent manner. Poster presentation, 2014, Hiroshima, Japan

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

2. 実用新案登録

3. その他