厚生労働科学研究費補助金（B型肝炎創薬実用化等研究事業）
革新的な動物モデルや培養技術の開発を通じたHBV排除への創薬研究
分担研究報告書（平成 25 年度）

# The discovery of host restriction factors required for HBV entry (infection) into mouse cells.

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研究要旨：
There is no immunecomptent 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. 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. NTCP overexpression rendered HepG2 cells permissive to HBV infection, in this year we tried to establish HBV infectivity in mouse hepatocytes by the overexpression of human NTCP.

## A. 研究目的 Aim

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 Method

In order to establish immune-competent HBV small animal model system we are aiming to first discover the human restriction host factors that limit HBV infectivity to only human and Chimpanzee hepatocytes. Last year, we used induced pluripotent stem cells (IPS) cells. These cells were previously shown to pass into 4 different stages until they differentiate into human hepatocytes (Definitive endoderm, Hepatic specification, Immature hepatocytes, Mature hepatocytes.) We aimed on identifying the stage of differentiation that is permissive for HBV infection, and by comparing the gene expression in this stage and the previous non permissive stage, we aimed to identify the

receptor required for HBV entry.

However, sodium taurocholate transporter (NTCP) was found last year to be the receptor recquired for HBV entry.

We then changed our aim from identifying the HBV entry receptor, to analyse if human NTCP can induce HBV infection of mouse hepatocytes.

To reach this target we used the following:

1- Establishment of mouse hepatocyte cell line permissive to HBV replication.

In-vivo primary mouse hepatocytes were previously reported to support HBV replication, however primary hepatocytes undergo senescence and death when cultured in-vitro. Hence we used primary like immortalized mouse hepatocytes by SV40-LT Oncogene. We then assayed HBV replication in these cells using the following:

HBV genome expressing plasmid is transfected into the obtained cell clones and HBV replication was assayed by the amplification of nuclease resistant HBV-DNA packaged in HBV-core particles by real-time PCR.

2- HBV-infection into immortalized mouse hepatocytes.

-HBV virus inoculum was derived from the medium supernatant of HepG2-4A5 cells. The supernatant was concentrated with PEG, and was used for infection.

Myc tagged Human NTCP was cloned from primary human hepatocytes, Myc tag is added at carboxy terminus and cloned into lenti virus vector and intoduced into mouse hepatocytes. The expression of human NTCP was confirmed by western blot. HBV infection was then performed, and infected cells were assayed for HBV infectivity by the amplification of nuclease resistant HBV-DNA packaged in HBV-core particles by real-time PCR.

**（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 result

HBV replication by the transfection of HBV-DNA was successfully established in immortalized mouse hepatocytes confirming that no block is present at the replication level of HBV in these cells. Human NTCP expression was confirmed by western blot analysis and its distribution to the cell membrane was also confirmed by immunofluorescence. However, infection of HBV particles into these cells was not successfully established, suggesting the presence of other host restriction factor required for HBV entry into mouse cells.

We then moved into the identification of host factors required for HBV life cycle in human HepG2 cells. Stable HepG2 cells expressing human NTCP and efficiently infected with HBV were established. We treated these cells

with siRNA libraries targeting several membrane proteins followed by HBV infection after 2 days. We then screened for the membrane proteins required for HBV entry which might act as another HBV receptor, this work is still undergoing.

We also screened for host factors required for HBV replication using AD38.7 cells. Since kinases are involved in many signaling pathways, and protein functions. We screened 500 human kinases for its function on HBV replication. We identified 4 kinases that are required for HBV replication, and 5 kinases that significantly suppress HBV. One of these kinases, TSSK2, it showed an efficient suppression of HBV infection and replication. Using interferon non-responsive cells we proved that the suppressive function of this kinase on HBV is interferon independent. We are now working on the identification of the mechanism by which TSSK2 suppress HBV.

## D. 考察 discussion

The permissiveness of mouse hepatocyte to HBV replication confirmed that there is no block at the replication level to HBV in mouse cells. However the lack of infection by HBV particles into human NTCP expressing cells suggested the presence of a still unknown host factor required for the establishment of HBV infection into mouse hepatocytes. This host factor may be another entry receptor, or another internal factor suppressing the early steps of HBV infection. Using siRNA library screening, we are currently trying to identify human host factors required for HBV life cycle, and its effect on HBV infectivity when overexpressed in human NTCP expressing mouse hepatocytes.

## E. 結論 conclusion

Mouse hepatocytes were susceptible to HBV replication, however HBV entry was not observed even after the expression of human NTCP receptor, suggesting the lack of another factor required for HBV entry. We are aiming to identify host factors affecting HBV entry and/or replication that might be required for HBV infectivity in mouse hepatocytes.

## F. 研究発表

1. 論文発表

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***5.*** **Aly HH**, Watashi K, Chayama K, Wakita T. Functional screening of human kinases interacting with Hepatitis B virus life cycle. ***The 36$^{th}$ annual meeting of the molecular biology society of Japan. Poster presentation, 2013, Kobe, Japan***

## G. 知的所得権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他