Development of cell culture system on the basis of adult stem cells for modeling of HBV infection

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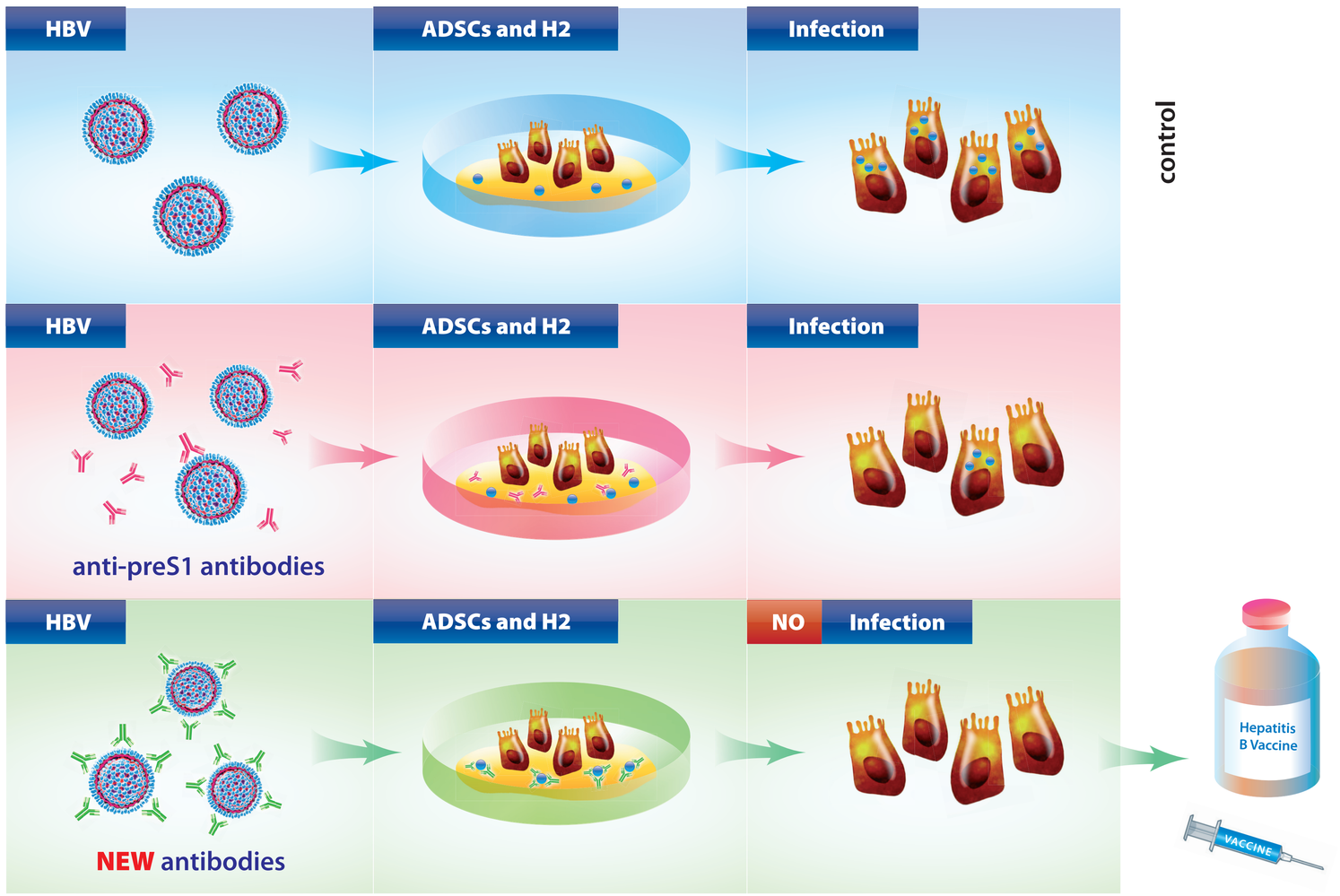
Introduction

Hepatitis B virus (HBV) infection remains a major global health problem and the leading cause of liver cancer worldwide.

Hepatitis B virus (HBV) infection remains a major global health problem and the leading cause of liver cancer worldwide. Efforts to elucidate the mechanisms by which HBV infects hepatocytes and causes liver damage have been hampered by the strict virus host range and a lack of suitable cell culture and animal models of HBV infection / replication. Relatively efficient infection by HBV is well documented for only humans, chimpanzees and tupaias *(Tupaia belangeri)* and, in cell culture, for primary hepatocytes from these hosts. For reasons that are not clear, infection of these primary hepatocytes and some established cell lines (e.g., HepaRG) with HBV has produced poor viral replication and low viral yields and has suffered from poor reproducibility. Therefore, the development of new HBV infection model to facilitate this area of research remains urgent. The present study has established and elucidated a novel HBV infection / replication *in vitro* model system based on adipose-derived stem cells (ADSCs) differentiated into human hepatocyte-like cell line.



Preparation of HBV particles for infection of ADSCs and H2

HBV particles used for infection were obtained from HepG2.2.15 cell line stably expressing the integrated virus. To evaluate the synthesis of HBV mRNAs in these cells, the total RNA was extracted from infected cells at 6-th day post-infection. The presence of viral antigens HBs and HBe were detected by ELISA (Fig.1).Finally, viral particles were visualised using transmission electron microscopy (Fig.2). As was detected by real-time PCR, the obtained HBV titre was $2{,}53 \times 10^8$ genome equivalents/ml.

Fig.1. **ELISA analysis of HBs and HBe.**

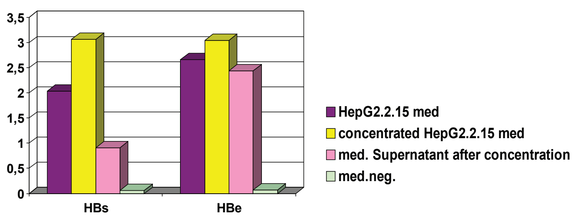
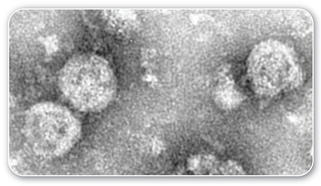


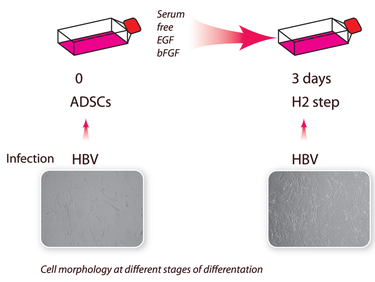
Fig.2. **electron microphotography of secreted HBV particles.**



Generation and evaluation of HBV infection-sensitive cells (ADSCs and H2)

ADSCs were isolated from human adipose tissues, propagated and characterized as multipotent stem cells. Then cells were cultured under pro-hepatogenic conditions according to Talens-Visconti (2006) generating stage H2 (Fig.3). Total RNAs were isolated from infected cells and subjected to HBV specific RT-PCR by random hexamer-directed synthesis of cDNA (TaqMan Reverse Transciption kit, Applied Biosystems) (Fig.4).

Fig.3. **Generation of H2 from ADSCs.**



Cell morphology at different stages of differentation

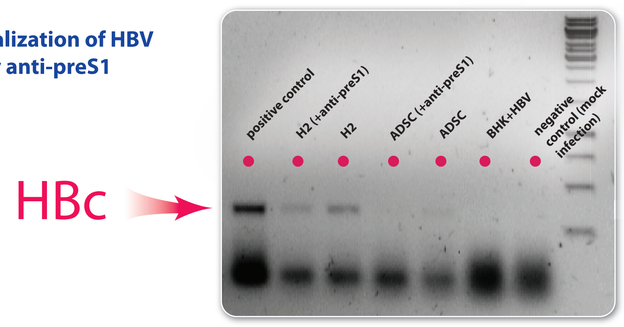
Fig.4. **Quantitation of HBc in infected cells by RT-PCR and real time RT-PCR.**

Cells	Newly synthesized HBV RNAs in infected cells as detected by RT-PCR	Real-time RT-PCR quantitation of HBc RNA prer 12-well
ADSC (Invitrogen)	-	4669.5
ADSC	+	1.78×10^4
H2	+++	9.27×10^4

Neutralization of HBV infection by anti-preS1 antibodies

In order to show specificity of HBV infection, virus neutralization experiments were performed. Monoclonal anti-preS1 antibodies (MA18-7) were used for neutralization of HBV stock before infection. The quantitative evaluation (mRNA per cell) of HBV transcripts (pgRNA and HBs mRNAs) were performed by real-time PCR (not shown). As was detected by PCR in agaroze gel, the inhibition of infection was observed for ADSC and H2 cells (Fig.5).

Fig.5. **Neutralization of HBV infection by anti-preS1 antibodies.**



Conclusions

Here we demonstrate that human ADSCs and H2, the cells of the first stage towards hepatic differentiation, have a potential to be infected by HBV.

Perspectives

- The novel HBV infection/replication model will be optimized to evaluate the replication capacity of various HBV mutant forms by direct infection. Thus, the established infection system will serve as a new tool for antiviral drug and vaccine prototype screening and provide means for studying the viral-host interactions and viral entry and uncoating – the unknown steps of HBV life cycle.
- The cells diferentiated to stage H2 will be adjusted for commercial purposes. For this reason, several H2 optimization steps have already been performed in the terms of division speed and infection efficiency. The resultant product, named 4bHHI, holds the potential as a cost-efficient and easy-to-use cell culture for HBV research.

Aknowledgements

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