Development of Antiviral Strategies for Treatment of Chronic *Hepatitis B*

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Key words: *hepatitis* B/*hepatocellular carcinoma*/treatment/chronic hepatitis/cytokines.

Introduction

Hepatitis B virus (HBV) causes acute and/or chronic hepatitis and is associated with hepatocellular carcinoma (HCC). There are currently 300 million HBV carriers worldwide. The establishment of an *in vitro* cell culture system for HBV infection is very important because it is an essential tool for the development of new antiviral strategies against hepatitis B virus infection and for studying the molecular events in viral replication. Historically, the major obstacle in the study of HBV has been the inability to infect animal model system due to strict virus-host range. It has been suggested that the tissue and species specificity of HBV infection may be due to the preferential attachment of the HBV envelope proteins onto the plasma membrane of human hepatocytes that lead to viral entry and replication. Many potential cellular receptors have been shown to interact directly or indirectly with the viral envelope proteins. The direct involvement of human Annexin-V, a calcium dependent phospholipid - binding protein in the initial step of HBV infection has been reported (Gong et al, 1999).

Materials and Methods

Chang liver and HepG2 cells were infected with serum containing Hepatitis B virus. The culture medium was modified to optimise the *in vitro* infection process and the cells cultured under standard cell culture conditions. Viral DNA was performed using the phenol chloroform lysis method and PCR performed with published primers. The PCR product was visualised after electrophoresis of ethidium bromide stained agarose gel. Paraffin embedded blocks from hepatitis B virus positive heptocellular carcinoma cases were sectioned and stained for cytokine detection by standard immunohistochemical methods.

Results and Discussion

The aim of this study is to establish an *in vitro* cell culture system that will continuously produce sufficient HBV by episomal replication. To facilitate the penetration and internalization of HBV, the expression of Annexin-V was enhanced using dexamethasone and by transfection of two liver cell lines, Chang liver and HepG2 cells.. The effect of upregulation of Annexin-V on susceptibility to HBV infection was investigated via immunofluorescent staining with antibodies to the viral envelope protein. Similarly, modification of the culture medium was found to increase the susceptibility of Chang cells to HBV infection and HBV replication as detected by immunofluorescence and via PCR amplication of cccDNA. The detection of cytokines such as IL-10 suggests that this immunosuppressive cytokine could play a role in the inability of the host to eliminate the malignant cells. IL-18 was found to be expressed in HCCs.

Conclusions

A cell culture system for studying the cellular changes following hepatitis B virus infection was accomplished. Results indicated the production of IL-18 in the tumour microenvironment associated with hepatitis B virus chronic inflammatory state.

Benefits from the study

This study has allowed the use of a cell culture system to study the cellular changes following hepatitis B virus infection. Therapeutic targets blocking the signals transduced by the virus could be potential drugs for the treatment of chronic hepatitis B. The detection of cytokine production provides insight to the use of cytokines as immunomodulators for treatment of chronic hepatitis B.

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I·R·P·A INTENSIFICATION OF RESEARCH IN PRIORITY AREAS

Research funded by the Intensification of Research in Priority areas (IRPA) - Supported by IRPA RM-7 (1996-2000), Cycle 1997, Grant 06-02-04-0016.