

# HBV-INDUCED MICROENVIRONMENT REDUCES PDC RESPONSE TO TLR9 STIMULATION

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## Abstract

### Objectives

Hepatitis B virus (HBV) is a non-cytopathogenic virus with DNA genome. It causes an acute hepatitis that may develop into chronic hepatitis B, liver cirrhosis and hepatocellular carcinoma. HBV is described as “stealth virus” as it possesses various mechanisms of interference with immune recognition.

We investigated the effect of HBV on activation of plasmacytoid dendritic cells (pDCs), as they are a main producer of interferon  $\alpha$  (IFN $\alpha$ ). IFN $\alpha$  secretion is induced by toll-like receptor 9 (TLR9) activation, yet this pathway is strictly regulated. One of the TLR9 pathway negative modulators is miRNA146a, which silences the TLR signalling proteins. Importantly, miRNA146a is active in the RNA-induced silencing complex (RISC), which binds the target mRNA and silences it. Mature miRNA-RISC can be sorted into extracellular vesicles (EVs) and transported to surrounding cells.

Therefore, we analysed, whether HBV infection affects the quantity of miRNA-146a secreted from hepatocytes, and whether the secreted miRNA146a affects the pDC function.

### Methods

Levels of HBV antigens and IFN $\alpha$  were measured using ELISA. Quantity of intracellular and extracellular miRNA-146a was analyzed by qPCR and digital droplet PCR, respectively. Transfection of miRNA inhibitors was performed with XMAN nanoparticles.”

### Results

HBV-producing hepatocytes, used as a model of chronic infection, expressed higher levels of intracellular and extracellular miRNA-146a than non-infected controls. The supernatant from the HBV-producing hepatocytes did not activate pDC cell line Gen2.2.. However, Gen2.2. cells exposed to the supernatant from HBV-producing cells secreted lower amounts of IFN $\alpha$  upon TLR9 agonist CpG-A treatment. Importantly, inhibition of miRNA-146a in HBV-producing hepatocytes, restored the IFN $\alpha$  production in Gen2.2. cells.

### Conclusions

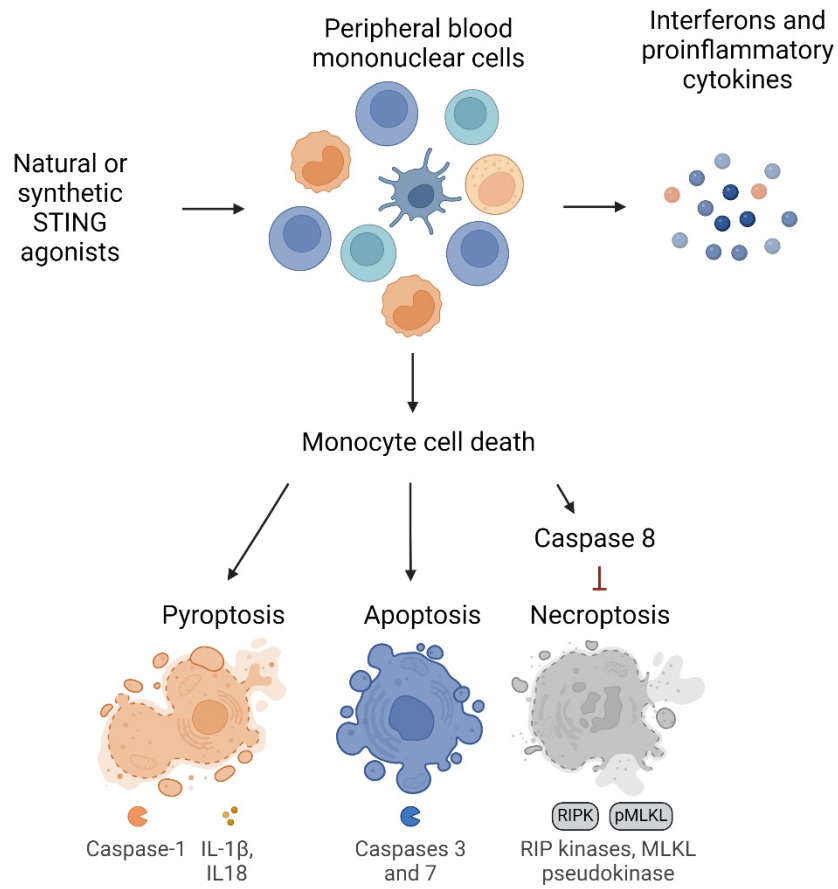
High amount of miRNA146a produced by the HBV-producing hepatocytes is transported likely via the EVs to pDC-derived cells, where it negatively regulates TLR9 signalling. Therefore, miRNA146a is an important factor in HBV-mediated immunomodulation, and could be a promising target for the chronic hepatitis B therapy.

### keywords

Chronic HBV, miRNA-146a, pDC, TLR9 signaling, extracellular vesicles

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