

Session: EV030 Viral infection & disease

**Category: 1b. Viral hepatitis (incl antiviral drugs, treatment & susceptibility/resistance, diagnostics & epidemiology)**

22 April 2017, 08:45 - 15:30  
EV0581

**Analyses of HNF4 alpha interactions with the hepatitis B virus genome by chromatin immunoprecipitation (ChIP)**

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**Background:** Hepatitis B virus (HBV) remains a serious global health problem. Thus far there is no reliable treatment which can completely eliminate the infection. Antiviral therapy strategies targeting viral transcription could potentially help to resolve chronic HBV infections. HBV transcription is one of the essential steps in the viral life cycle, and it is dependent on hepatocyte-encoded coactivators and transcription factors including liver-enriched nuclear receptors. The hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ) transcription factor is essential for viral biosynthesis in the liver. The purpose of this study is to determine the binding profile of HNF4 $\alpha$  to the HBV enhancer/promoter sequences in HepG 2.2.15 cells by ChIP.

**Material/methods:** For ChIP analysis, HepG 2.2.15 cells were grown under standard conditions. Cells were plated and used at 80% confluency (approximately  $5 \times 10^6$  cells per 10cm plate). Crosslinking and nuclei preparation was performed as described (The truChIP Chromatin Shearing Reagent Kit, Covaris). Chromatin was sheared by sonication (Covaris S220 sonicator) on ice to generate cellular chromatin fragments of about 200 - 300 bp. Chromatin samples were incubated with anti-HNF4 $\alpha$  (Cat. No: ab94748 and sc6556, Abcam and Santa-Cruz, respectively) and anti-H3K27Ac polyclonal antibody (Cat. No: ab4729, Abcam) prior to immunoprecipitation with ChIP-grade Protein A/G Magnetic Beads (Fisher Scientific). Reversal of cross-linking followed by the elution of the protein/DNA complexes was performed using the QIAquick PCR Purification Kit (Qiagen). Human

cytochrome P450 (CYP26A1), sulfotransferase (SULT2A1) and complement (C3) promoter sequences were used as HNF4 $\alpha$  positive control targets to validate any observed binding of HNF4 $\alpha$  to HBV enhancer/promoter sequences. The following sets of primers were used to examine the HBV regulatory sequence elements within the HBV genome: Enhancer I/X-gene promoter, enhancer II/nucleocapsid promoter, polymerase gene open reading frame, presurface antigen promoter, major surface antigen promoter. For qPCR, Fast SYBR Green Master Mix (Applied Biosciences) was used on a 7500 Real-Time PCR machine (Applied Biosciences). Results were analyzed by using the  $\Delta CT$  method. Standard deviations are indicated. Statistical differences were analyzed by Student's *t* test.

**Results:** Our results indicate that occupancy rates of HNF4 $\alpha$  at HBV promoters was highest within the enhancer I/X-gene and enhancer II/nucleocapsid promoter regulatory elements which are critically important for the transcriptional regulation of the pregenomic RNA (Figure I).

**Conclusions:** HNF4 $\alpha$  is an important transcription factor which regulates HBV transcription and replication in HepG2.2.15 cells. Consequently HNF4 $\alpha$  might be a potential target for the development of new antiviral strategy targeting the modulation of viral transcription.

**Figure I: Occupancy rate of HNF4 $\alpha$  at HBV promoters**

