**Conclusions:** The replication activity of hepatitis B virus in HBeAg-positive group was higher than another two groups. The virus replicated actively and liver function damage by no means synchronization occurrence. Total HBV DNA in serum samples combined with intrahepatic cccDNA can better monitor the anti-viral therapy effect and prognosis.
**Abbreviation:** Alanine aminotransferase (ALT), total bilirubin (TBIL), hepatitis B e antigen (HBeAg)

### PP-009 Short hairpin RNA with 2–4 bases mismatch inhibiting B and C genotype HBV in vitro

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Until recently, only alpha interferon and nucleotides (analogue) were available for the treatment of HBV. However, the low efficacy, undesirable side-effects, and drug- resistance remain the major obstacles in treating HBV infection, the need for alternative therapeutic measures has provided the impetus to develop novel therapeutic reagents for inhibiting HBV replication.
RNAi has proven to be very powerful in inhibiting HBV replication by cell culture and mouse model studies. The present study all designed perfect match siRNAs to target HBV, as we all known that HBV exist as quasispecies, so, it is essential to study inhibitory effect of mismatched siRNAs on target HBV; here, we study the inhibitory effect of 2–4 bases mismatch shRNAs on HBV.
**Objective:** To assess the inhibitory effect of shRNAs with mismatching with target sequence on B and C genotype HBV.
**Methods:** shRNA and pHY106-BHBV or pHY106-CHBV cotransfected HepG2 cells, the supernatant of the culture at 24 h, 48 h, 72 h, 96 h and 120 h after cotransfection was collected and frozen in –20°C for examining HBsAg and HBeAg by ELISA; 72 h after cotransfection, the total RNA of cells was exstracted and reverse-transcripted, then detecting the level of HBsAg mRNA by RT-PCR; 72 h after cotransfection, cells were lysated, HBV replicative intermediate from HBV core particles was exstracted for southern blot.
**Results** 1. HBsAg and HBeAg expression were inhibited obviously by shRNA458 and shRNA635, the inhibitory effect was detectable at 48 h after cotransfection, the peak time was 72 h, the most inhibitory ratio was approximately 80% and 50% in HBsAg and HBeAg; there was no difference was observed about the the inhibitory action of shRNA458 and shRNA635 on B or C genotype HBV; 2. shRNA458 and shRNA635 had the similar inhibitory action on the production of HBsAg mRNA in B and C genotype HBV at 72 h after cotransfection, the inhibitory ratio was 60–70%; 3. HBV replicative intermediate from B and C genotype HBV was inhibited obviously by the two shRNAs also.
**Conclusions** shRNA458 and shRNA635 have powerfully inhibitory effect on the expression of HBsAg and HBeAg, HBsAg mRNA and HBV replication although 2–4 bases mismatch between shRNAs and target HBV, so shRNA458 and shRNA635 are two powerful tools for inhibiting HBV.

### PP-010 3D structural and dynamic features of Protein-x of hepatitis B virus

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**Background and Aim:** The protein-x of Hepatitis B virus (HBx) interacted directly or indirectly with host factors to modulate cell signaling pathways and would have suggested being oncogenic. Though many functions have been associated with protein-x, the nature of this protein is not well disclosed. Full understanding of the biological role of this protein will require a complete knowledge of its structure. Here we present a structural model to picture the structure of HBx using computational approaches along with the available experimental data.
**Methods:** Protein-x was translated based on its genome which we have sequenced previously. Bioinformatics tools were employed for searching Hidden Markov Model and conserved motif(s). SSpro program was performed to determine the secondary structure of the protein and the ProtScale program was used to provide several predefined amino acid scales. Our model was constructed using 1jfua as a template by LOMETS server. Optimization of the model was carried out by the molecular dynamics simulated method.
**Results:** Analysis of the amino acid sequences of HBx by pfam database clarified a large conserved domain and motif search showed a Trans-activation region for protein-x. PSI-Blast revealed not more than 26% identity against the PDB database.
Refined model included a stable folding with a coiled motif and three helical domains with two antiparallel β-sheets. The model was tested using molecular dynamics simulation.
**Conclusion:** Although there is limited knowledge about protein-x of hepatitis B virus, here we modeled a unique 3D structure for the first time. The model was tested for validation and dynamic simulations in water. This model can lead to better understanding of the function of protein-x and its interaction with the cellular proteins that could be useful for the design of selective inhibitors as a target in treatment.

### PP-011 An undamaged bulge of ε is essential for initiating priming of DHBV reverse transcriptase

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Hepatitis B virus (HBV), the type member of the *Hepadnaviridae*, is responsible for infections that cause B-type hepatitis. The small enveloped HBV virus replicates via reverse transcription of an intermediate RNA-the pregenomic RNA (pgRNA), and initiates by protein-priming to the epsilon (ε) stem-loop on the pgRNA. The ε hairpin consists of a lower stem, an apical loop, an upper stem, and a bulge region which serves as template of priming, i.e. 3 or 4 nt *de novo* DNA synthesis. ε is essential for the encapsidation of the pgRNA and initiation of reverse transcription. Both events are mediated by binding of reverse transcriptase (P protein) plus cellular chaperones to ε RNA. However, within ε, little information is known which structural elements are indispensable for priming.