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Título: Persistence of hepatitis delta virus infection in absence of ongoing HBV replication

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Introduction

The hepatitis Delta virus (HDV) is a small RNA defective virus that can only become infectious in the presence of hepatitis B virus surface antigens (HBsAgs). It is acknowledged that the presence of the two virus increases the risk of HCC (Hepatocellular carcinoma) and fulminant liver failure.

During chronic HBV infection, the integration of HBV DNA in hepatocytes genome may occur and become a viable source of HBV envelope proteins production, necessary for the maintenance of HDV persistent infection. As so, we believe that HDV could actually persist in the absent of ongoing HBV replication as long as HBsAgs are supplied from integrated DNA.

Aim

To determine whether mRNAs transcribed from integrated HBV DNA lead to translation of the HBV envelope proteins that efficiently support the assembly and infectivity of HDV virions.

Methods

A total of five pairs of HBV-infected human liver tissues and matching HBV-induced HCCs were used to isolate HBV DNA through the amplification by nested PCR of 3 overlapped fragments, F1, F2 and B, respectively. The DNA fragments were cloned using pCR®II-TOPO® vector and the consensus sequences found for each tissue were used as references to design oligonucleotides for integrant-derived mRNAs coding for the envelope proteins amplification. Since those mRNAs must have a 3'-end insertion of host sequences ensuring the polyadenylation at ~1830nt, a strategy of RT-PCR, followed by Nested PCR, cloning and

sequencing procedures, allowed us to discriminate the HBV integrant-derived mRNAs sequences from the mRNAs which result from HBV replication.

Results

At least seven different clones for each of the three DNA fragments amplified were analyzed. Based on the overlapped obtained sequences, we artificially achieved the entire genome HBV sequence for the five pair of liver tissues studied. With those, we designed a protocol to amplify the mRNAs coding for the HBV envelope proteins. The expected structure for functional envelope proteins should have the 5′-UTR, the entire L coding sequence, and most of 3′-UTR of HBV sequence. In integrated sequences, at 3′ host sequence it is presumed to have the poly(A) signal and the part of poly(A) addition site remaining after the cleavage at ~1830nt. With the exception of one pair of liver samples, we observed that the polyadenylated RNAs obtained from normal liver tissues, were mainly HBV replication derived ending at ~1930, On the other hand, the mRNAs of matching HCCs sequences, were verified to be mainly integrands that were variable in length and coding sequence, in and between the studied samples.

Conclusion

After the detection of promising integrant-derived mRNAs for HBV envelope proteins the ongoing study aims to construct LMS vectors for *in vitro* infection of primary human hepatocytes to determine for the first time whether integrated HBV envelope proteins can ensure the efficient assembly of HDV virions.