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DNA sequences homologous to hepatitis C virus (HCV) in the extrachromosomal circular DNA in peripheral blood mononuclear cells of HCV-negative subjects

Key words: **Hepatitis C virus (HCV), 5'-Non-coding region (5'-NCR), Human genome, Extrachromosomal DNA, Circular DNA, Pattern of methylation**

Research Summary

- We continued to follow previous experimental findings of DNA sequences that are substantially homologous to the corresponding 5'-NCR RNA sequence sections of Hepatitis C virus (HCV); these DNA sequences are present in the whole DNA extracted from the PBMCs of HCV negative subjects.

- This study focuses on the extrachromosomal circular DNA (ecc DNA) extracted from PBMCs of HCV negative subjects.

- We tested for patterns of methylation within the HCV homologous sequences of the ecc DNA fraction.

- The ecc DNA share of the total cellular DNA ranges from about 4 to 20%.

Innovation points I

- These ecc DNAs harbor sequences which are essentially homologous to the 5'-non- coding region (5'-NCR) of the Hepatitis C virus.

- Depending on the primers used for the home-made PCR these sequences range up to 320 bp.

- Testing these sequences with a pre-PCR restriction enzyme digestion protocol with four restriction enzymes (RE): the RE are inhibited when methylation (s) are present at specific cytosine residues within their targeting sites.

- The results revealed unique methylation patterns for each of the subjects analyzed; therefore, they may represent individual DNA-based methylomes. The results after pre-PCR hint at an epigenetic phenomenon being imminent with the 5′-NCR HCV DNA contained in these ecc DNAs

Innovation points II

- In contrast, BLASTn alignment searches of published HCV 5'-NCR sequences with human genome (HG) databases revealed only sequence segments of up to 36 bp of the 5'-NCR. Their locations on chromosomes vary, probably due to different/newer versions of the chromosomal DNAs available in the HG databanks.

- It is strange that the ecc-DNA fractions have not yet been considered explicitly as part of the total DNA when used for whole genome sequencing (WGS) of the human genome project. Since the ecc-DNA fractions also contain other virus-specific sequences, checking for possible inaccuracies due to ignoring the ecc-DNA seems to be necessary.