中文題目:肝癌細胞株不同C型肝炎病毒量之感染細胞之微小RNA表現

英文題目: The microRNA Expression of Hepatocellular Carcinoma Cells with High and Low Hepatitis C Viral Load

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Background: The hepatitis C virus (HCV) infection and replication varies among individual hepatocytes in chronic HCV infection by identifying hepatocytes with different HCV viral RNA. We have previously established a Fluorescence-activated cell sorting (FACS) protocol to study the effects of different intracellular viral loads in HCV-infected cell populations. We have found that cells with the different viral load presented different levels of DNA damage and capacities for damage-repair ability. In the present study we aimed to further study the RNA (miRNA) expression more detail on different viral load cells.

***Methods*:** We used the JFH1-EYFP viral florescence intensity to sort the high and low viral load cell. The next generation sequence-RNA sequence was used to clarify the mRNA and miRNA gene network between HCV-high and HCV-low infected cells of the HCC cell line. Venn diagram summarizing the probe sets that were differentially expressing between the Huh7.5.1 versus each differential viral load cell population and miRDB and miRTar databases were used to predict HVL and LVL/S2 unique miRNA target genes and to analyze gene functional annotation by DAVID system. Ingenuity pathway analysis (IPA) software was used to re-construct the connection between miRNA and target genes.

Results: We investigated mRNA and miRNA expression profiles in different viral load cell populations by analyzing the NGS dataset and miRNA microarray dataset. After analyzing the NGS dataset and miRNA microarray dataset, of the significant transcripts, two miRNA were unique for the LVL/S2 cells and nine miRNA unique for the HVL. We verified them by q-PCR and data confirmed the array data expression level. Gene annotation results indicated that HVL 9 miRNA target genes are cluster with “cell death” and “viral reproductive process”. The LVL 3 miRNA tatget genes are cluster with “cell motility” and “response to stress”. The KEGG pathway analysis further determined the genes are all about the inflammation-associate pathway in high viral load cells. However, the low-viral load cells presenting high percentage of genes involved in cancer pathway and cell adhesion molecules pathways. IPA software found SPINK1 is the major target gene which by miR-10a and miR-215 overexpression in LVL cells. The vimentin was significantly overexpressed in low viral loads cells both in WB data and ICC staining results indicating the trend of epithelial-to-mesenchymal transition which was regulated by the SPINK1.

Conclusions: By our established cell sorting protocol, we found the genes enriched cell death, viral reproduction, and cell inflammation pathways in HVL cells; and genes enriched cell stress, cell

motility, and cancer pathways in the long time cultured LVL/S2 cells. The significantly overexpressed miRNAs on LVL cells were associated with SPINK1 expression which may be associated EMT-associated proteins alternation. The miRNAs predicting target gene profiles provide more significant information on the HCV-associated hepatocarcinogenesis.