論文の内容の要旨

論文題目　Genetic Alterations of Hepatitis Virus Oncogenes in Association with Treatment Resistance and Carcinogenesis

（治療抵抗性と肝発癌に関与する肝炎ウイルス癌遺伝子変異）

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Hepatocellular carcinoma (HCC) is currently the fifth most common cancer and the second leading cause of cancer related mortality worldwide. Up to 80% HCC is related to chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. In recent years, increasing attention has been given to viral mutations that potentially governing hepatitis virus related carcinogenesis though the pathogenic mechanisms remained unclear. In our study, we focused on two virus oncogenes (HBV, HBx; HCV, core) and investigated the association between genetic alterations of these two proteins and patients' treatment response and carcinogenesis.

Part 1. The Characteristic Changes in Hepatitis B virus X region for Hepatocellular Carcinoma: a Comprehensive Analysis Based on Global Data

In this study, we sought to clarify potential HCC characteristic mutations in HBx from HBV genotype C infected patients and the distribution of those mutations in different disease phases and genotypes. HBx sequences downloaded from an online global HBV database were screened and then classified into Non-HCC or HCC group by diagnosis information. Data of patient age, gender, country or area, and viral genotype were also extracted. Logistic regression was performed to evaluate the effects of mutations on HCC risk. In total 1115 human sera originated full length HBx sequences (HCC: 161; Non-HCC: 954) across 29 countries/areas were extracted from the downloaded 5956 HBx sequences. Genotype C occupied 40.6% of Non-HCC (387/954) and 89.4% of HCC (144/161). We found 16 nucleotide positions showing significantly different distributions between the genotype C HCC and Non-HCC groups. Logistic regression demonstrated that mutations A1383C (OR: 2.32, 95% CI: 1.34-4.01), R1479C/T (OR: 1.96, 95% CI: 1.05-3.64; OR: 5.15, 95% CI: 2.53-10.48), C1485T (OR: 2.40, 95% CI: 1.41-4.08), C1631T (OR: 4.09, 95% CI: 1.41-11.85), C1653T (OR: 2.58, 95% CI: 1.59-4.19), G1719T (OR: 2.11, 95% CI: 1.19-3.73), and T1800C (OR: 23.59, 95% CI: 2.25-247.65) were independent risk factors for genotype C HBV-related HCC, presenting different trends among individual disease phases. In addition, several genotype C HCC risk

mutations pre-existed, even as major types, in early disease phases with other genotypes. This study revealed that mutations associated with HCC risk were mainly located in HBx transactivation domain, viral promoter, protein/miRNA binding sites, and the area for immune epitopes. Furthermore, the signatures of these mutations were unique to disease phases leading to HCC, suggesting molecular counteractions between the virus and host during hepatocarcinogenesis.

Part 2. Amino acid 70 Substitutions in Genotype 1b HCV Core Protein and Responses to PEG-IFN/RBV Treatment

HCV core protein, adding to the viral nucleo-capsid fomation, has multiple functions such as regulation of host-cell transcription, apoptosis, cell transformation, and lipid metabolism. It was also shown to be potentially oncogenic in transgenic mice. Recently, a single amino acid 70 substitution [Arg (70W) to Gln (70M)] of core protein was reported to be associated with PEG-IFN/RBV treatment failure and hepatocarcinogenesis in HCV genotype 1b infected patients. While it was presumed that 70M strain could resist the PEG-IFN/RBV treatment, molecular genetic insights into the viral properties still remain limited. In this study, we performed a core 70W/M-specific realtime PCR to examine the existence of HCV 70W/M quasispecies among HCV Gt 1b-infected patients. Also we monitored the dynamic changes of core 70W/M during antiviral treatment, analyzing those from patients with different treatment responses/periods, and examined clinical and host factors associated with 70M in Japanese patients with HCV Gt 1b infection.

We found that, before treatment, 25 (74%) patients were coinfected with 70W/M. Ratios of co-existing 70M and 70W did not show significant differences in the resistance to PEG-IFN/RBV treatment while a higher 70M ratio was significantly associated with higher possibility of NVR ($P<0.01$). Interestingly, relapsers predominantly with 70W at baseline showed 70M predominance at the early stage of relapse while afterwards returning to 70W predominance. Moreover, the predominant 70W sequence after relapse was derived from 70W sequence observed at baseline. Univariate analyses exhibited that 70M ratio was associated with IL28B polymorphism and platelet count at baseline. Multivariate logistic regression showed 70M ratio was an independent predictor for NVR while IL28B was the strongest predictor for SVR.

Thus, we suppose that the core 70M strain was rather a product selected by the complex interactions between virus and host immune system than the one merely by the PEG-IFN/RBV treatment. A further understanding of these HCV variants is likely to be of increasing importance in order to identify the most appropriate treatment for infected individuals. Novel therapeutic strategies targeting the 70M strain capable of replicating under low selection pressure may serve to eradicate HCV infection without relapse.