Title: Determination of Hepatitis C (HCV) Genotypes and Drug Resistances By a Efficient and Cost-effective Sequence Analysis Method: One cDNA synthesis, two assays

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Abstract

A more efficient, high specific and cost-effective RT-PCR sequencing method has developed for a correct HCV genotype and study the natural genetic variability and drug resistance within HCV non-structure region.

Infection with hepatitis C virus (HCV) frequently leads to chronic hepatitis with an increased risk for the development of liver cirrhosis and liver cancer. HCV is classified into eleven major (designated 1-11), many subtypes (designated a, b, c, etc.), and about 100 different strains based on the sequence heterogeneity. In Sweden, the genotype distribution was different from that in studies from other parts of the world, with a lower frequency of genotype 1b and a higher frequency of genotype 1a and 3a. HCVgenotype differences affect responses to antiviral therapy, for exemple, patient infected with genotype 1 responds only 50% to PEG-IFN-á and ribavirin treatment in 48 weeks and approximately 80% of patients infected with HCV genotypes 2 and 3 treated with PEG-IFN-á plus ribavirin in 24 weeks achieve a sustained virological response. It has been suggest that the therapeutic strategy should be different for genotype 1 (and 4-6) and genotypes 2 and 3, respectively. Therefore, determination of HCV genotype and antiviral resistance are important and must be performed after the diagnosis of chronic hepatitis C, in order to provide the most effective treatment for HCV infected patients.

To identify a correct genotypes and mutations that confer drug resistance to HCV protease inhibitors in untreated patients, especially mutations involving R155K substitution. We have recently developed a “One cDNA synthesis and two assays” RT-nestPCR method where we sequenced the HCV NS5b region for the genotyping and protease gene for determination of resistance mutations. cDNA was synthesis using random primer and PCR primers were designed from the NS5b region for genotyping and NS3 regions for determining mutations which covers known 10 protease resistance in NS3, including R155K and V36M. Sequences were then analyzed and phylogenetic tree was made for genotypes according to alignment, and identification of resistance substitutions in the NS3 protease was performed by Seqscape software. RT-nestPCR assay was successful in samples containing >100IU/mL HCV RNA. The accuracy of this method has been validated by QCMD (Quality Control for Molecular Diagnosis, UK). Our method represents a more efficient in identifying mixture of genotypes (2k/1b, 2a/2c/2i), specific and reliable method for differentiation between all genotypes and subtypes, economic and is useful in study natural genetic, mutations and polymorphism within HCV NS3 protease region.

This simple, more efficient, specific, low-cost and reproducible method can be used as a routine diagnostic and should also be useful to monitor resistance directly during treatment. The results will be integrated in discussions of therapeutic and diagnostic strategies in the Nordic regions. Such diagnostic method has yet been developed in Sweden.

Biography

Dr. Hong Yin has been working in RNA viruses for many years and has great knowledge within the field of RNA viruses. She graduated in China and obtained her PhD at Uppsala University, Sweden. She is currently working as a senior molecular virologist at Uppsala University Hospital and focus on developing new molecular diagnostic methods for hepatitis C genotyping and antiviral drug resistance.

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