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Codon Usage as a Possible Source of Sofosbuvir Genetic Resistance Bias in HCV Patients Infected with Different Genotypes

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Abstract

Hepatitis C virus (HCV) is responsible for liver chronic infections that in a high percentage of cases lead to cirrhosis and hepatocellular carcinoma. In the past, treatment consisted on a combination of pegylated interferon plus ribavirin. However, since 2012 a new wave in treatment options appears with the new family of drugs targeting viral components, the so-called direct-acting antivirals. Now we have a battery of drugs directed against viral protease (NS3/4A), polymerase (NS5B), and viral NS5A protein. Sofosbuvir, a nucleotide-analogue directed against viral polymerase is one of the most effective drugs against HCV. However, pathways for resistance acquisition are obscure. One mutation can be considered either a resistant-associated polymorphism (RAP) or a resistant-associated substitution (RAS) depending on the virus genotype. Thus, L159F is considered as a RAP in genotype 1b but as a RAS for genotypes 1a and 3. Previous studies propose differences in the NS5B structure from different genotypes as the leading cause for these genotype differences. However, other reasons could be triggering these disparities. In this short communication we analyzed sequences from two different cohorts of HCV-infected naïve patients. From these data we show that codons coding for L159 and C316 amino acids are genotype-dependent and changes in these positions could be acquired differentially depending on the relative abundance of the corresponding tRNAs.

Commentary

Hepatitis C virus (HCV) is responsible for chronic infections in almost 200 million people worldwide. HCV-positive patients are at high risk of developing cirrhosis and hepatocellular carcinoma [1]. HCV is a positive strand RNA (RNA (+)) virus showing high replication rates in the absence of proof-reading activity. HCV worldwide distribution together with its high error genome replication have led to a great HCV sequence diversity showing at least seven genotypes and different subgenotypes [2]. Furthermore, the quasispecies nature of HCV genome distribution is shown as a population of different but closely related genomes even when different samples obtained from the same infected patient are compared [3].

Sofosbuvir is a nucleoside-analogue inhibitor of the HCV polymerase (NS5B). This drug is one of the most important actors in the new HCV-treatment interferon free era. Resistance to Sofosbuvir has been related to the appearance of mutations in NS5B, but the types of changes as well as the pathways to acquire them are poorly understood. Recently, it has been published an article about the potential resistance pathways for Sofosbuvir (SOF) [4]. Donaldson & colleagues described the appearance of low-frequency substitutions at conserved NS5B amino acids in 224 subjects who failed treatment. Resistance-associated substitutions (RAS) are mutations that are selected during treatment in subjects who failed treatment. RAS mutations are L159F for genotypes 1a and 3, and V321A for genotype 3. Authors also describe resistance-associated polymorphisms (RAP) that are changes present at baseline in subjects who failed treatment. RAP mutations are L159F and C316N for genotype 1b. In any case, the number of patients carrying these mutations was low. The pathway for SOF resistance mutation acquisition seems to show a high genetic barrier but the underlying mechanism is not understood. Both RAS and RAP changes were proposed to introduce structural changes into the catalytic pocket of the enzyme directly related to the resistance phenotype. In order to know the prevalence of these changes in sequences derived from naïve HCV-positive patients we have analyzed the presence of L159F and C316N mutations in two sets of HCV sequences from naïve HCV-infected patients described in Legrand-Abravanel et al. [5] and Bartels et al. [6]. From these analyses we have found that codons coding for residues 159 and 316 are genotype-dependent. This data has allowed us to establish a new hypothesis about the pathway of acquisition of resistance mutations.

A total of 888 sequences were analyzed, including 542 of genotype 1a, 250 of genotype 1b, and 96 of other genotypes (25 of genotype 2, 25 of genotype 3, 31 of genotype 4, and 15 of genotype 5). RAP mutations L159F and C316N were detected in 18 out of 250 genotype 1b sequences (7.2%), and in 29 out of 250 1b sequences (11.6%), respectively. RAS mutations (L159F for G1a and G3, and V321A for G3) were not detected in any sequence of these two sets. Furthermore, sequences of genotypes 3, 4, and 5 showed wild type residues Leu, Cys, and Val at positions 159, 316, and 321, respectively. Only one sequence of genotype 2 showed the mutation V321L instead of V321A substitution.

Codon usage varies among different species and contributes to

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Table 1: Codon usage in positions 159 and 316 of NS5B in different genotypes and subtypes.

	159		316
L	ctc (G1a,G2b,G3) ctt (G1b,G2a)	**C**	tgt (G1a,G2b) tgc (G1b,G2a,G3)
F	ttc ttt	**N**	aat aac

In bold, one-letter code for amino acids. Three letter code was used for the codons. Genotypes are between brackets.

translation regulation [7]. HCV genotypes can be distinguished by the codon used in some positions including those related to antiviral resistance (Table 1). Most common codons (> 90% sequences) codifying for L159 residue are ctc (for G1a, G2b, and G3), and ctt (for G1b, and G2a). Most common codons for C316 residue are tgt (for G1a, and G2b) and tgc (for G1b, G2a, and G3). Therefore, nucleotide change needed for L159F substitution is only one transition, from ctc to ttc for G1a, G2b, and G3, or from ctt to ttt for G1b, and G2a. Nucleotide changes needed for C316N substitution are one transition and one transversion, from tgc to aac in genotypes 1b, 2a, and 3, and from tgt to aat in genotypes 1a and 2b.

Focusing on genotype 1b, where L159F and C316N are considered RAP [4], nucleotide changes needed for C316N mutation are one transition and one transvertion (tgc to aac) , whereas only one change (ctt to ttt) leads to L159F mutation. However, L159F and C316N mutation frequencies in genotype 1b are 7.2% and 11.6%, respectively. Therefore, the highest mutation frequency corresponds to the mutation with the highest number of nucleotide changes. On the other hand, L159F mutation was not detected in HCV sequences derived from naïve patients of genotype 1a for which this change was classified as a RAS. This result was expected because the sequences analyzed were isolated from naïve patients, but this mutation is a one-step mutation as for genotype 1b. Genetic barrier is lower than for C316N that needs two different nucleotide substitutions. Furthermore, C316N is considered RAP whereas L159F is a RAS, indicating that C316N is a more common mutation than L159F. Once again, although C316N needs more nucleotide changes than L159F, the former is more common.

Why Occurs this?

Codons associated with these positions are genotype-dependent, as we mentioned before. Thus, L159 codon ctc is representative of G1a sequences whereas ctt is representative of G1b. These differences in the codon usage in positions related to resistance could be in the basis of the higher genetic barrier detected for L159 in addition to the structural changes proposed previously [4]. Higher abundance of Phe codon ttt than ttc could explain that L159F mutation is RAS for G1a and RAP for G1b. In other words, high prevalence of cellular tRNA ttt codon makes L159F mutation more prevalent in G1b where the change is ctt to ttt. Under the same conditions (high prevalence of cellular tRNA ttt codon) G1a virus would need two transitions (from ctc to ttt). Therefore, if the Phe codon usage is biased G1b viruses would need only one change (from ctt to ttt) whereas G1a viruses would need two (from ctc to ttt). Actually, codon bias plays an important role in controlling a multitude of cellular processes, including translation efficiency and protein folding [8]. Also, codon bias has been extensively described in the dual system virus-host [9].

Recently, Selitsky and co-workers have described that small RNAs derived from tRNAs (5' tRHs) were significantly increased in humans with chronic viral hepatitis [10]. These 5' tRHs are the product of RNase activity of Angiogenin on cellular tRNAs [11]. Because these 5'tRHAs are more abundant in patients carrying active viral hepatitis, relative concentration and proportions of tRNAs could be altered. All these data focus on relative abundance of tRNAs as one of the determinants of genetic resistance barrier to SOF. In any case, further experiments should be performed to establish the relationship between resistance pathways and abundance of cellular tRNAs.

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