Characterization and structure prediction of the 5' untranslated region from Hepatitis C Virus Colombian isolates

Santiago Alvarez-Cardenas[1], Camila Lopez-Osorio[1], Rodrigo Ochoa[2], Maria-Cristina Navas[1]

1. Grupo Gastrohepatología, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.
2. Biophysics of Tropical Diseases, Max Planck Tandem Group, Universidad de Antioquia, Medellín, Colombia.

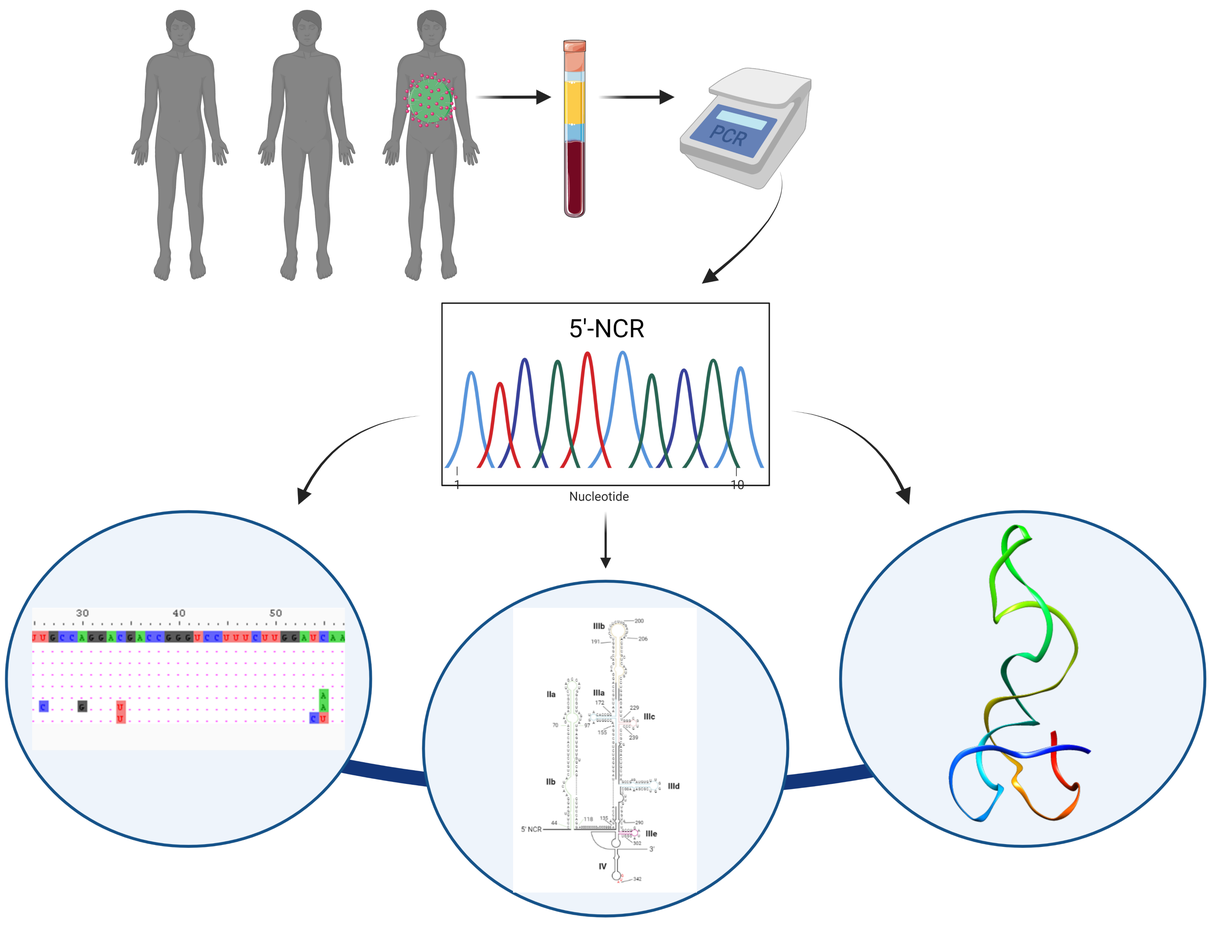
INTRODUCTION

The genome of the Hepatitis C Virus (HCV) is a single stranded positive sense RNA flanked by 5’ and 3’ untranslated regions (UTR). The 5’UTR has a relevant role in translation of the HCV polyprotein driven by the internal ribosome entry site (IRES). This structured region contains four domains (DI-DIV) from which DII and DIII have the main role in the interaction to the ribosome, and therefore for initiation of translation. Furthermore, DII presents two subdomains (basal DIIa, and apical DIIb) while DIII consists of 6 stem-loops (DIIIabcdef).

AIM

In this study we aim to characterize seven sequences of 5’UTR of HCV and identify the substitutions or insertions that could modify the secondary structure and, therefore, the tertiary structure of the IRES, which could affect the interaction with the translational machinery.

METHODS



A total of seven sequences of HCV were obtained from a previous study carried out in Medellin, Colombia, six of genotype 1 (GT 1) and one of genotype 4 (GT 4). The secondary structure was predicted using RNAFold v2.3, while the tertiary structure using RNAComposer v1.0. The DII and DIII were modeled individually considering similar Protein Data Bank (PDB) structures available. The obtained models were then compared to the template structures and the implications of the mutations in the structural conformation of the IRES were analyzed.

RESULTS

We found 1 insertion and 10 substitutions, mainly transitions on the HCV sequences. Moreover, most of the mutations were in DIII (7/11). Some transitions had a major effect on the DIII conformation in certain subdomains by favoring the formation of G-C bonds and giving a higher structural stability. Certain genotypes may be prone to present more mutations that affect the tertiary conformation, as seen with the GT, 4d subgenotype, sequence. Domain II appear to be conserved among the sequences. Meanwhile, DIIIabc tend to vary more than DIIId and DIIIe (Fig 1, Table 1).

Fig. 1. Reference (UP) vs S1 (DOWN) DIIIabc.

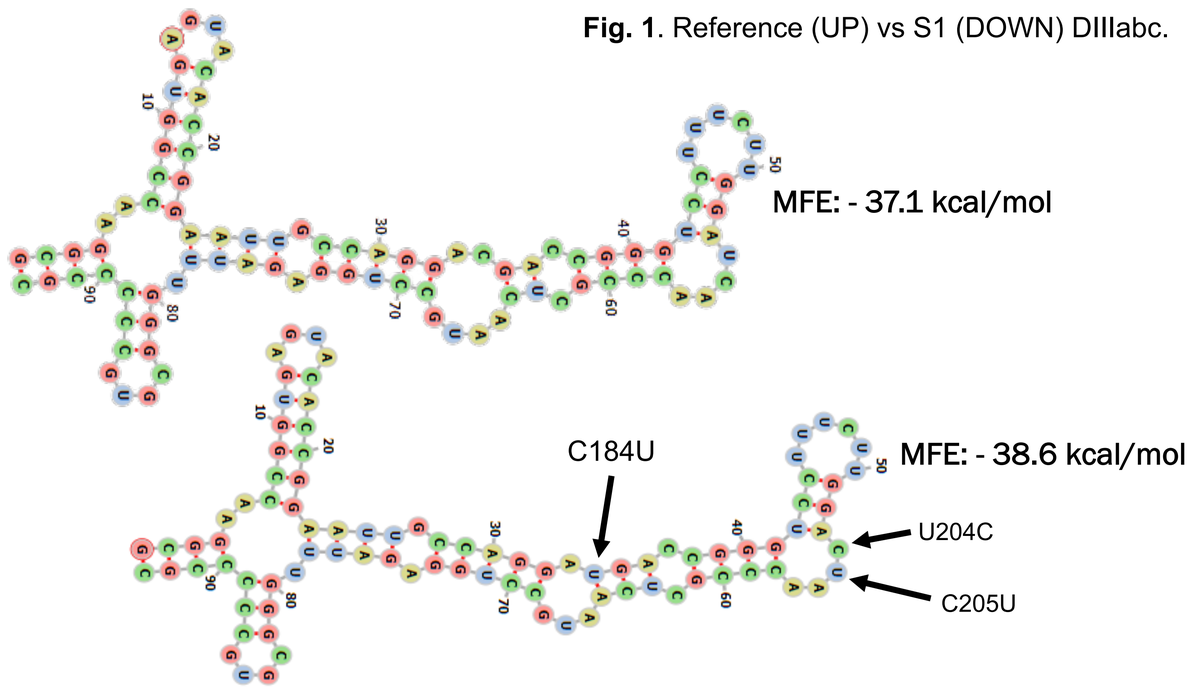


Table 1. HCV Sequences and characteristics

ID	Genotype / Subtype HCV	Seq Length	Coverage (vs Ref D90208) nt-nt	Mutations HCV 5'NCR (DII, DIII)
S1	1b	280 nt	1-284	C184U, U204C, C205U
S2	1b	220 nt	63-282	A73
S3	4d	211 nt	92-302	C104U, G107A, U176C, U180C, C184U, C205A, U221C, G225A
S4	1b	222 nt	63-285	-
S5	1b	221 nt	63-283	A73
S6	1a	243 nt	63-306	-
S7	1a	230 nt	73-302	C205A

CONCLUSION

The mutations described in 5’UTR HCV a three-bond for a two-bond nucleotide did not affect the secondary structure of loops. However, a deeper analysis on the frequency and location of these mutations is required to understand the phenomena that shapes the IRES conformation.

A docking analysis with the 40S ribosomal subunit to assess the affinity with the sequence 3 (GT 4) compared with GT 1 specimens and reference sequence is in progress.

ACKNOWLEDGEMENT