ARFp expression as a biomarker of progression of hepatic disease in hepatitis C virus infection

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Introduction

- 79 million people are infected with Hepatitis C virus (HCV) and per year 400,000 people die from diseases related to HCV infection (WHO, 2017);
- In chronic hepatitis C, there is progression of liver fibrosis, which can develop cirrhosis and hepatocellular carcinoma (HCC) (WHO, 2017);
- Fibrosis can be classified in a five - level severity scale, from F0, without fibrosis, to F4, which is cirrhosis (Nguyen-Khac E and Capron D, 2006);
- The HCV genome has a main open reading frame (ORF) that originates a precursor polyprotein, but in the alternative reading frame -2 / + 1, an additional protein, ARFp (Alternative Reading Frame protein) is synthesized (Xu et al., 2001);
- ARFp is expressed during natural HCV infections and stimulates specific immune responses, but it is not essential in viral replication. Its function is still unclear, but may be related to carcinogenesis (Fiorucci et al., 2007);
- Previous studies have shown a higher prevalence of anti-ARFp antibodies in patients with cirrhosis compared to patients without cirrhosis, but did not consider the different stages of fibrosis within the non-cirrhotic group (Dalagiorgou at al., 2011).

Objectives

- Detection of anti-ARFp antibodies in HCV positive patients with chronic infection with different degrees of fibrosis (F0 to F4);
- Detection of anti-ARFp antibodies in HCV patients with cirrhosis (F4) with and without HCC symptoms.

Materials and Methods

Plasmids

3 plasmids were donated by contributors of Hellenic Pasteur Institute:
•core genotype 1a;
•ARFp genotype 1a;
•ARFp genotype 3a.

Expression tests

The plasmids were transformed into E.coli BL21 (DE3) cells to express using IPTG. Comparing diferents temperatures (15ºC, 30ºC and 37ºC), incubation time (3h, 8h, 16h and 24h) and buffer with or without urea.

Purification

The proteins were purificated using nickel column by affinity chromatography in the AKTA Purifier 100 (GE). Western-blot was performed in order to confirm that it was no contamination among plasmids, using antibody anti-histidine and antibody anti-IgG with peroxidase.

Elisa

The proteins will be used to sensitize ELISA plate and the serum of patients will be tested for core (positive control) and ARFp.

Patients

260 patients with HCV genotype 1 grouped by fibrosis level:
- 128 were classified as fibrosis F0/F1/F2;
- 132 as F3 or F4.

Results

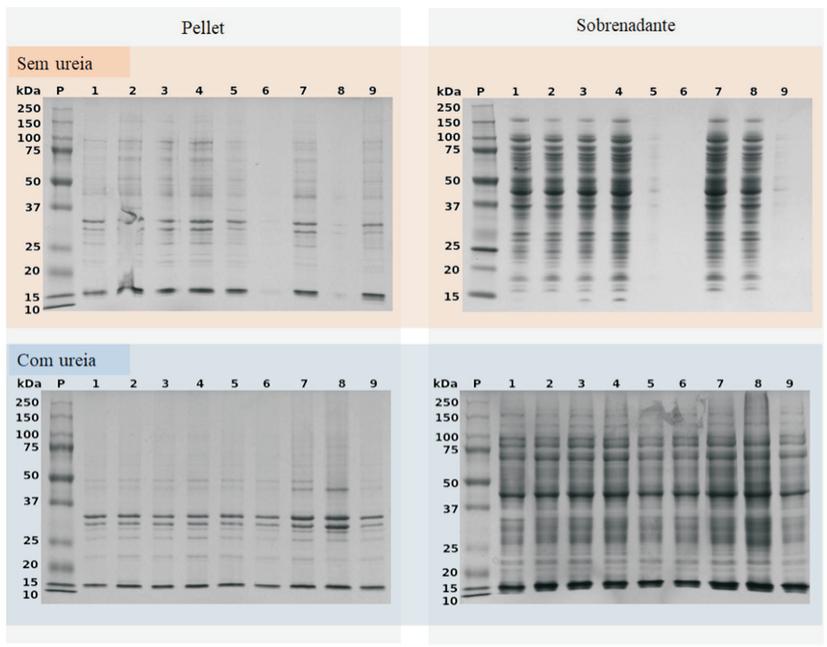

Figure 1. Different conditions of core protein (17 kDa) expression. Legend: (P) = Marker kDa, 1 = 3 hours/15 °C, 2 = 8 hours/15 °C, 3 = 16 hours/15 °C, 4 = 24 hours/15 °C, 5 = 3 hours/30 °C, 6 = 8 hours/30 °C, 7 = 16 hours/30 °C, 8 = 24 hours/30 °C e 3 hours/37 °C.

Table 1. Proportion of anti-Core antibodies in each patient group.

Patients	n	Core +
F0	4	4 (100%)
F1	74	73 (99%)
F2	54	51 (94%)
F3	51	51 (100%)
F4	83	81 (98%)
HCC	13	11 (85%)
Total	279	271 (97%)
Blood donors	28	0

Table 2. Proportion of anti-ARFp 1a antibodies in each patient group.

Patients	n	ARFp 1a +	p
F0	4	2 (50%)	
F1	73	15 (21%)	0.8184
F2	51	9 (18%)	
F3	51	20 (39%)	0.8561
F4	81	34 (42%)	
HCC	11	10 (91%)	
Total	271	90 (33%)	
Blood donors	28	0	

Table 3. Comparison of the proportions of anti-ARFβ antibodies in patients with mild (F0/F1/F2) and severe fibrosis (F3/F4)

Patients	n	ARFp 1a +	p
F0/F1/F2	128	26 (20%)	0.0004
F3/F4	132	54 (41%)	

Table 4. Comparison of the proportions of anti-ARFP antibodies in cirrhotic patients with and without HCC.

Patients	n	ARFp 1a +	p
F4 without HCC	128	26 (20%)	0.0004
F4 with HCC	132	54 (41%)	

Conclusions

- The best condition for protein expression with IPTG induction was 3 hours at 37 ° C, sonication in buffer with urea and purification from the supernatant portion.
- There was no difference between the antibodies proportions of individuals anti-ARFp 1a + between levels F1 and F2, as well as between levels F3 and F4, but when comparing the groups with mild fibrosis (F0 to F2) and advanced fibrosis (F3 and F4) , the proportion of anti-ARFp antibodies was significantly higher in patients with increased liver damage.
- A significantly higher antibodies proportion of individuals with anti-ARFp 1a + in the group with F4 level and HCC were observed than in the F4 group and without HCC.
- These results suggested the presence of this protein is related to progression of liver disease. We conclude that the anti-ARFp antibody can be used as a biological marker of progression of liver fibrosis in patients infected with HCV.

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