ARFP EXPRESSION AS A BIOMARKER OF PROGRESSION OF HEPATIC DISEASE IN HEPATITIS C

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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

- To detect anti-ARFp antibodies in HCV positive patients with different levels of fibrosis as a biomarker of progression of hepatic disease in Hepatitis C;
- To determine the proportion of HCV patients infected with genotypes 1, 2 and 3 with anti-ARFp antibodies according the fibrosis level (F0 to F4);
- To determine the proportion of HCV patients infected by genotype 1 with anti-ARFp antibodies with HCC versus non-HCC patients with cirrhosis (F4).

MATERIALS AND METHODS

Plasmids

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

Stocking

The Plasmids were transformed into E.coli DH5α.

Expression

The plasmids were transformed into E.coli BL21 (DE3) cells to express using IPTG.

Expression tests

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

It was realized 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.

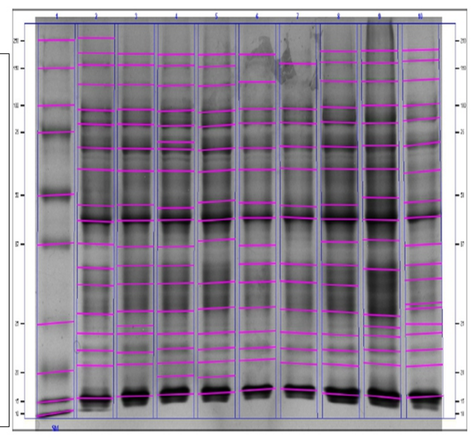
Patients

108 patients with HCV genotype 1 grouped by fibrosis level:
- 4 F0/F1;
- 10 F2;
- 67 F4.
- 10 F1;
- 16 F3;

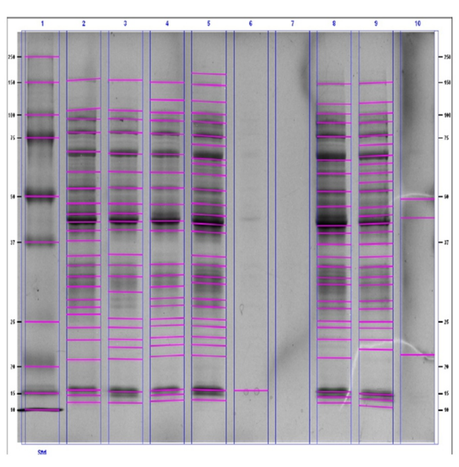
RESULTS

Expression test:

With urea:
1) pattern;
2) 15ºC 3h;
3) 15ºC 8h;
4) 15ºC 16h;
5) 15ºC 24h;
6) 30ºC 3h;
7) 30ºC 8h;
8) 30ºC 16h;
9) 30ºC 24h;
10) 37ºC 3h.

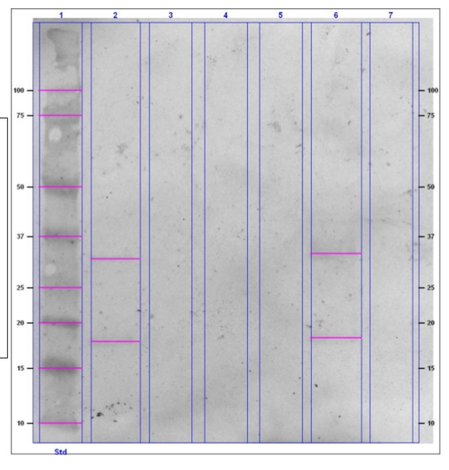


Withot urea:
1) pattern;
2) 15ºC 3h;
3) 15ºC 8h;
4) 15ºC 16h;
5) 15ºC 24h;
6) 30ºC 3h;
7) 30ºC 8h;
8) 30ºC 16h;
9) 30ºC 24h;
10) 37ºC 3h.



Western Blot:

1) Pattern;
2) Non filtrated material;
3) Filtrated mterial;
4) Not bound proteins;
5) 1st fraction of elution;
6) 2nd fraction of elution;
7) Negative control.



Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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