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Squamous cell carcinoma antigen-1 (SERPINB3) polymorphism in chronic liver disease☆

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Abstract

Background. The serpin squamous cell carcinoma antigen (SCCA, SERPINB3) has been found over-expressed in primary liver cancer and at lower extent in cirrhosis and chronic hepatitis. A novel SCCA-1 variant (SCCA-PD), presenting a single mutation in the reactive centre (Gly351Ala), has been recently identified (rs3180227).

Aim. To explore SCCA-1 polymorphism in patients with HCV infection as single etiologic factor and different extent of liver disease.

Methods. One hundred and fourty-eight patients with chronic HCV infection (45 chronic hepatitis, 53 cirrhosis, 50 HCC) and 50 controls were evaluated. SCCA-1 polymorphism was studied by restriction fragment length polymorphism and confirmed randomly by direct sequencing. Circulating SCCA–IgM complex was determined by ELISA.

Results. SCCA-PD was detected with higher frequency in cirrhotic patients (45.3%, odds ratio = 2.62; 95%CI 1.13–6.10, $p = 0.038$) than in patients with chronic hepatitis or in controls (24.4% and 24%, respectively). Intermediate figures were found in hepatocarcinoma (36.0%). SCCA–IgM in serum was lower in patients carrying SCCA-PD than in wild type patients and the difference was statistically significant in cirrhotic patients (mean ± S.D. = 117.45 ± 54.45 U/ml vs. 268.52 ± 341.27 U/ml, $p = 0.026$).

Conclusions. The newly identified SCCA-PD variant was more frequently found in liver cirrhosis, suggesting that patients carrying this polymorphism are more prone to develop progressive liver fibrosis.

Keywords: Fibrosis; Liver disease progression; SCCA polymorphism; Serpins

1. Introduction

The serpin squamous cell carcinoma antigen (SCCA) has been found over-expressed in hepatocellular carcinoma (HCC) [1–3], being also detectable in the liver of patients with cirrhosis and chronic hepatitis, although the extent of expression was lower than that observed in neoplastic livers [4]. In addition, increasing amounts of circulating SCCA–IgM complex have been identified in cirrhotic patients at higher risk of liver tumour development [5]. By direct mRNA sequencing a new SCCA-1 (SERPINB3) variant (SCCA-PD) has been identified in our laboratory, presenting the $351_{G\rightarrow A}$ mutation in the reactive centre of the protein [1] (GenBank accession number: AY190327, SNP identification: rs3180227). Since the mechanism of protease inhibition by serpins involves a profound change in conformation, initiated by interaction of the protease with the reactive centre of the serpin [6], the single aminoacid change detected in the reactive loop of

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SCCA-PD might alter the anti-protease activity of this serpin variant.

Aim of the present study was to investigate the distribution of SCCA-1 polymorphism in patients with different extent of liver disease.

2. Patients and methods

2.1. Patients

A total group of 148 Caucasian patients with chronic HCV infection as single etiologic factor were studied. Patients were selected from those regularly followed up in our Institution and included 45 cases with histologically proven chronic hepatitis, 53 with liver cirrhosis and 50 cirrhotic patients with HCC. Diagnosis of cirrhosis was defined on clinical, biochemical, ultrasonographic and/or histologic criteria, while the diagnosis of HCC was based on the presence of hepatic focal lesion(s) detected by liver ultrasound and confirmed by computed tomography and/or magnetic resonance as imaging techniques. The final diagnosis was confirmed by histopathologic analysis on ultrasound-assisted fine-needle biopsy, when indicated. Baseline characteristics of the patients included in the different groups are reported in Table 1. The exclusion criteria were the presence of co-occurring HBV and/or HIV infections, age >80 years, chronic alcohol abuse, autoimmune liver diseases and metabolic disorders. Fifty blood donors were used as controls (31/19 M/F; mean age ± S.D.: 43.1 ± 11.9).

Peripheral blood mononuclear cells (PBMCs) and the corresponding serum samples were obtained under informed consent and stored at −80 °C until use.

2.2. SCCA-PD polymorphism

Genomic DNA was isolated from PBMCs by standard procedures, as previously described [7]. All DNA samples were coded to ensure blinding of patient information and genotyping was performed by technicians who were unaware of the histological data.

The newly identified SCCA-PD polymorphism was assessed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

The amplification reaction was performed in a total volume of 100 μl containing 100–500 ng of genomic DNA, 200 μM dNTPs (Roche Diagnostics, Branchburg, NJ), 1.5 mM $MgSO_4$ (Invitrogen, Carlsbad, CA), 0.2 μM primer sense (5′-AAGCATGATTGTGTTGCTGCC-3′), 0.2 μM primer antisense (5′-ATCTACGGGGATGAGAATCTGC-3′), 10X High Fidelity PCR Buffer (Invitrogen, Carlsbad, CA), 1.5 U Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA).

The protocol for the Thermal Cycler 480 (PerkinElmer, Norwalk, CT) was as follows: 94 °C for 5 min, followed by 55 °C for 45 s, and 68 °C for 100 s (1 cycle); 94 °C for 45 s, followed by 55 °C for 45 s, and 68 °C for 100 s (32 cycles) and the last cycle of 94 °C for 45 s, followed by 55 °C for 45 s, and a final extension step (68 °C for 5 min). The amplified product was digested with BsmI (New England Biolabs, Beverly, MA) at 65 °C for 90 min and then inactivated for 20 min at 80 °C, since the $351_{G\rightarrow A}$ mutation creates a BsmI restriction site. After enzyme digestion and agarose gel electrophoresis, the G allele (wild type) was recognized by the occurrence of a 1222 bp fragment, while the C allele (mutated type) showed two bands of 1098 bp and 123 bp.

The genetic results were confirmed randomly by direct sequencing, using an ABI 310 automated DNA sequencer (Apply Biosystems, Foster City, CA), according to manufacturer's instructions.

2.3. SCCA–IgM immune complex

Circulating SCCA–IgM immune complex was determined in serum using an ELISA assay (Hepa-IC, Xeptagen S.p.A.,

Table 1
Baseline characteristics of the patients included in the different groups for the study

	Chronic hepatitis	Cirrhosis	HCC	Controls
Number of patients	45	53	50	50
Age (years, mean ± S.D.)	61.2 ± 11.4	63.8 ± 9.3	64.0 ± 10.8	43.1 ± 11.9
Gender (%)				
Male	19 (42.2)	32 (60.4)	38 (76)	31 (62.0)
Female	26 (57.8)	21 (39.6)	12 (24)	19 (38.0)
HCV genotype (%)				
1a	9 (20.0)	10 (18.9)	8 (16.0)	–
1b	27 (60.0)	30 (56.6)	29 (58.0)	–
2a–c	7 (15.5)	9 (16.9)	9 (18.0)	–
3a	2 (4.4)	4 (7.5)	4 (8.0)	–
Child-pugh class (%)				
A	–	21 (39.6)	19 (38.0)	–
B	–	22 (41.5)	24 (48.0)	–
C	–	10 (18.9)	7 (14.0)	–

HCC = hepatocellular carcinoma.