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Osteopontin in hepatocellular carcinoma: A possible biomarker for diagnosis and follow-up

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ABSTRACT

Recently osteopontin (OPN), a protein of the extracellular matrix, has generated in hepatocellular carcinoma (HCC) a significant interest as a prognostic factor. Aim of this study was to confirm, in liver tissues of subjects with HCV-positive HCC undergoing liver transplantation (RL, n = 10) and of donors (DL, n = 14), the increase of OPN plasma and tissue concentration, the OPN splicing isoforms expression profiling together with those of thrombin, and to evaluate a possible association between OPN measurements. Their association with Notch-1, IV-Collagen-7s domain, IL-6 and TNF-α were also evaluated. Real-Time PCR experiments and immunometric assay were performed. mRNA expression resulted higher in RL than in DL for all analyzed genes and several correlations were found between them. The more relevant association were between OPN-a and OPN-b ($p < 0.0001$), between thrombin and OPN-a ($p = 0.007$), between 7s-collagen and OPN isoforms ($p < 0.05$) and between Notch-1 with OPN-c ($p = 0.004$). Both OPN plasma and liver tissue extract concentrations were assessed confirming the trend observed at the mRNA level. An important association was found between OPN plasma and protein ($p < 0.0001$, $r = 0.96$) even splitting patients in DL ($p < 0.0001$, $r = 0.93$) and RL ($p < 0.0001$, $r = 0.96$). A reduction of OPN plasma levels was found at 6 months after transplantation. Considering MELD score as liver disease severity, the mRNA expression of our markers as well as of OPN plasma and tissue concentrations resulted increased as a function of clinical severity. Our results might be considered a useful starting point to validate OPN as a prognostic and diagnostic marker of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent causes of death in the world and its frequency is continuously increasing [1,2]. This disease develops predominantly in subjects with a history of hepatitis B (HBV) or C virus (HCV), but also in subjects with non-alcoholic fatty liver disease or with a history of chronic alcohol abuse [3]. Moreover, often it is difficult to have an early diagnosis of the disease due to the coexistence of inflammation and cirrhosis. Hepatocarcinogenesis is a multiphasic process that involves deep alterations to the cell genome including high cell proliferation. In particular, HCV-related HCC seems to be related to a higher frequency after hepatic resection, suggesting that this viral infection plays a key role in promoting cell proliferation as well as in metastasis [4]. Although the mechanism of HCV-induced HCC is not yet known, HCV represents the most common causes of death among HCC patients. Moreover, hepatocarcinogenesis can develop even in absence of cirrhosis and also in association with hepatic metabolic derangement. Several cytokines have been studied as possible actors in the liver disease progression and HCC development, and as early circulating biomarkers that can predict subjects at higher risk.

The identification of biomarkers that facilitate early detection and surveillance of HCC in high-risk patients could have, in fact, a significant influence on the mortality rate associated with this cancer. However, to date, few HCC biomarkers have adequate diagnostic performance in clinical practice. So, it becomes very important to know about new biomarkers that can be used to develop new prevention strategies to be used in these situations. Among the most promising biomarkers, osteopontin (OPN) seems to play a relevant role both for early diagnosis of HCC than on the mechanisms that drive oncogenesis [2]. OPN is a multifunctional calcium binding-phosphorylated-acidic glycoprotein of the extracellular matrix (ECM). It is expressed in several

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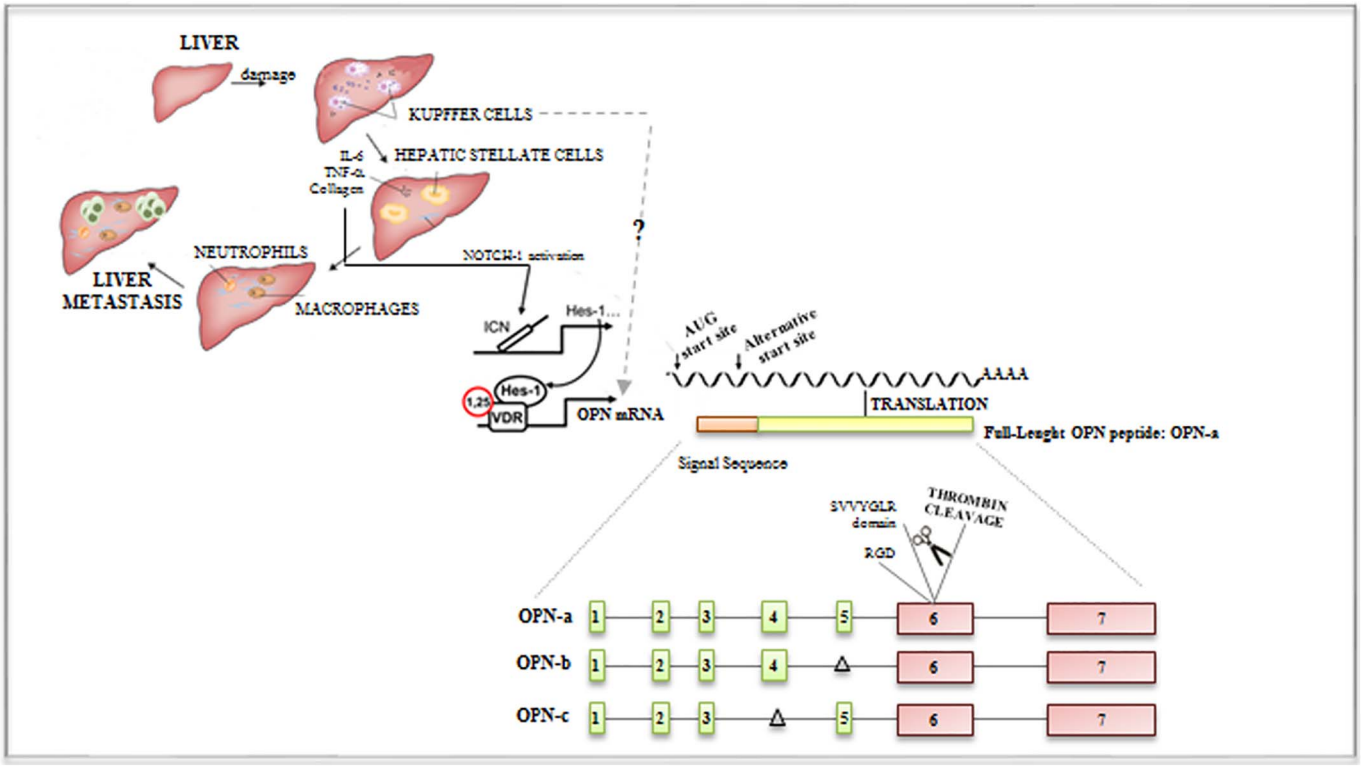

Fig. 1. Sequential steps in liver metastatic progression and osteopontin isoforms [the full-length isoform (OPN-a), OPN-b (lacks exon 5), and OPN-c (lacks exon 4)] cascade (modified by Yun Zhang & Xiao-Fan Wang, Nature Cell Biology 2015).

organs and it is involved in multiple biological cellular functions [5–7]. It is over-expressed in many different malignances and, its levels were found increased in many tumors and inflammatory processes [5,6,8,9]. In the last years it had been suggested as a prognostic marker in recurrent tumors and to date is recognized as a diagnostic marker [5,6,8,9]. Among the tumors in which it appears to be involved, OPN has aroused interest, especially in the HCC since recent studies have highlighted its important role in diagnosing liver tumors [10–12] even if still not yet clear. Moreover, different OPN isoforms have been identified and have been reported to be associated with cancerogenesis [13]. In humans, three isoforms were expressed differently: the full-length isoform OPN-a, the OPN-b and OPN-c (Fig. 1) [14–16]. Between these only OPN-a was extensively described in various cellular processes with a particular interest in the mechanisms of carcinogenicity. To date, the role in HCC of thrombin, the enzyme able to cleavage OPN, and of the three derived OPN isoforms has not been deeply evaluated. Recent data suggested OPN as the responsible gene in the activation of the Notch signaling pathway in HCC [17] which play a critical role during cell proliferation, differentiation but also during carcinogenesis [18].

Aim of this study was to confirm, in liver tissues of subjects with HCV-positive HCC undergoing liver transplantation and of donors, the increase of both OPN plasma and tissue concentration, the OPN splicing isoforms expression profiling together with those of thrombin, and to evaluate a possible association between OPN measurements so that plasma OPN might be used as a biomarker for diagnosis and follow-up. We also evaluated their association with Notch-1 and Type IV Collagen 7s domain, known to be indicators of tumorigenesis and proliferation of HCC.

2. Materials and methods

2.1. Patients selection and sample collection

The study was conducted in collaboration with the liver transplantation unit of the University of Pisa where the patients with hepatocellular carcinoma (HCC) were admitted for surgery after providing informed consent. In the current project were enrolled twenty-eight subjects: 14 subjects with HCV-related HCC undergoing liver transplantation (age 59.4 ± 1.8 years) and 14 donors (age 62.1 ± 17.3 years). Four patient with HCV- related HCC resulted diabetic, and for consistency of results were excluded from the analysis. The inclusion criteria were patients with histologically proved HCC and an age > 18 years. Exclusion criteria were the same as those reported in our previous studies conducted on the same patients [19,20]. Briefly, we excluded patients with: (a) non-HCC liver neoplasms, (b) acute liver failure, renal and cardiovascular diseases, (c) a model for end-stage liver disease (MELD) score 30 at transplantation, (d) a body mass index (BMI) > 30 kg/m^2, (e) autoimmune liver disease, hepato-pulmonary syndrome, (f) less than 6 months of alcohol consumption, (g) mental impairment, (h) porto-pulmonary hypertension. Donor and recipient clinical and biochemical characteristics are reported in Table 1. At the time of transplantation, patients were re-screened for compliance with the eligibility criteria and underwent laboratory tests. The MELD score at transplantation was derived as indicated elsewhere [21]. Approval was obtained from the Institutional Ethics Committee. The work has been carried out in accordance with the Code of Ethics of the World Medical Association (declaration of Helsinki) for experiments involving human subjects.

2.2. Tissue samples

The tissue samples were harvested during surgical procedures from the donor liver graft (DL) and from the recipient's liver (RL) explants. They were collected in RNAlater (Sigma-Aldrich, St. Louis, MO, USA) and stored at −20 °C. From the same patients blood samples were also obtained and stored at −80 °C. Follow-up procedures included in the recipient further blood withdrawal at 3 and 6 months after liver transplantation.