Epithelial cell adhesion molecule is a prognosis marker for intrahepatic cholangiocarcinoma

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ABSTRACT

Background: Recently, we identified a gene signature of intrahepatic cholangiocarcinoma (ICC) stroma and demonstrated its clinical relevance for prognosis. The most upregulated genes included epithelial cell adhesion molecule (EpCAM), a biomarker of cancer stem cells (CSC). We hypothesized that CSC biomarkers could predict recurrence of resected ICC.
Methods: Both functional analysis of the stroma signature previously obtained and immunohistochemistry of 40 resected ICC were performed. The relationships between the expression of CSC markers and clinicopathologic factors including survival were assessed by univariate and multivariable analyzes.
Results: Gene expression profile of the stroma of ICC highlighted embryonic stem cells signature. Immunohistochemistry on tissue microarray showed at a protein level the increased expression of CSC biomarkers in the stroma of ICC compared with nontumor fibrous liver tissue. The overexpression of EpCAM in the stroma of ICC is an independent risk factor for overall (hazard ratio = 2.6; 95% confidence interval, 1.3—5.1; $P = 0.005$) and disease-free survival (hazard ratio = 2.2; 95% confidence interval, 1.2—4.2; $P = 0.012$). In addition, the overexpression of EpCAM in nontumor fibrous liver tissue is closely correlated with a worst disease-free survival ($P = 0.035$).
Conclusions: Our findings provide new arguments for a potential role of CSC on ICC progression supporting the idea that targeting CSC biomarkers might represent a promise personalized treatment.

1. Introduction

Intrahepatic cholangiocarcinoma (ICC) is considered to be a therapeutic challenge and a public health issue. Indeed, although ICC incidence is increasing in all western countries [1], complete surgical resection is currently the only available curative treatment. After surgery, the 5-y survival rate of patients with ICC remains low ranging between 25% and 35% in

most of the series [2,3]. This poor prognosis is related to the high recurrence rate, especially during the first year after liver resection. Recently, we showed that ICC recurrences occurred in about half of the patients after surgery with curative intent, frequently during the first year and usually in the remnant liver only [4]. This major early recurrence rate and the high rate of patients diagnosed with satellite nodes in both lobes suggest that ICC is a disseminating parenchymal liver disease. The genomics and molecular mechanisms involved in the onset and progression of ICC are poorly documented. Many arguments based on both experimental data and the expression of specific markers indicated that ICC may originate from cancer stems cells (CSC) or hepatic progenitor cells (HPCs) [5]. CSC share functional characteristics with normal stem cells including the potential of self-renewal and pluripotency. Several reports argue about the role of CSC in tumor progression, tumor relapse after treatment, and resistance to chemotherapy. Accordingly, Sia *et al.* [6] demonstrated in a genome-wide analysis that their subgroup of progressive ICC was associated with a stem-like ICC signature. Recently, by combining laser capture microdissection and gene expression profiling, a gene signature of the tumor stroma (TS) in ICC have been established and associated with a clinical relevance on prognosis [7]. This genomic signature included 1073 nonredundant genes that significantly discriminate the TS from NFT. Interestingly, the most upregulated genes in the TS included epithelial cell adhesion molecule (EpCAM), a well-known marker of CSC in liver tumors [8].

To date, the precise functions of CSC in liver tumors remain poorly understood, particularly due to the lack of accurate cell surface markers that can be isolated on CSC. However, Ma *et al.* [9] showed that the presence of CSC was associated with a poor histopathologic grade and a worse survival including disease-free survival (DFS) in hepatocellular carcinoma (HCC).

The aim of the study was to determine whether the expression of CSC markers could predict recurrence after surgical resection of ICC.

2. Methods

2.1. Patient characteristics and tissue samples

Forty patients who underwent liver resection with curative intent for ICC at Rennes University hospital between January 1997 and August 2011 were studied. Only mass-forming type ICC, as defined by the Liver Cancer Study Group of Japan, were included and analyzed. Formalin-fixed paraffin-embedded blocks containing tumor and surrounding NFT were retrieved from the archives of the Pathology Institute of Rennes University. The histology of all tumors was reviewed and confirmed by two experienced pathologists and classified according to Union International against Cancer Control seventh edition. Clinical features were obtained from hospital charts. Data were collected on demographics (age, gender, and body mass index), viral status (hepatitis B virus and hepatitis C virus) and the presence of an underlying liver disease. After resection, the follow-up protocol included a clinical examination and a computed tomography scan every 3 mo during 2 y, then every 6 mo thereafter. The end of the follow-up was

set between January 1, 2013 and March 1, 2013, or at the time of death. The study protocol fulfilled national laws and regulations and was approved by the local ethics committee.

2.2. Data mining of ICC RNA profiles

RNA analysis was made by using the gene expression profiles that we established previously from the microdissected stroma of human ICC or fibrous tissue in the adjacent non-tumor liver tissue [7]. The full expression dataset was downloaded from the gene expression omnibus database (accession number, GSE45001). Gene set enrichment analysis was performed by using the Java tool developed at the Broad Institute (Cambridge, MA) as previously described [10].

2.3. Tissue microarray construction

To reduce the experimental variations and to standardize the results, immunohistochemistry (IHC) was made by tissue microarray (TMA). TMA design and construction were performed using TMADesigner software and a Minicore 3 tissue Arrayer (Excilone, VICQ, France) as previously described [7]. Briefly, after a hematoxylin–eosin staining, three representative areas of stroma from each ICC (TS) and of fibrous tissue from portal tracts areas in the surrounding nontumor liver (NFT) were selected by an experienced pathologist (B.T.) and were punched with a cylinder of 1 mm diameter before transferred to a TMA block. Thus, each tissue block (tumor and nontumor) was represented by three independent spots in the TMA. Subsequently, immunohistochemical studies were performed on 4-μm tissue sections of TMAs.

2.4. Immunostaining for EpCAM, CD44, and CD133

IHC was performed for CSC markers (EpCAM, CD44, and CD133). As described previously, 4-μm tissue sections of the TMAs were deparaffinized and immunostained using an automated Discovery XT immunostaining device (Ventana Medical System, Tuckson, AZ). The following primary monoclonal anti-mouse antibodies were used: EpCAM (1/50; eBiosciences, San Diego, CA), CD44 (1/200; ProMab Biotechnologies, Richmond, CA), and CD133 (1/200; ProMab Biotechnologies, Richmond, CA). Detection was performed using a streptavidin-biotin-peroxidase kit (OmniMap, Biotin-free DAB detection systems; Ventana Medical System). Staining results were independently scored by experienced pathologist (B.T.) in a blind manner. Staining intensity in the stroma was scored as follows: negative (0), mild (1), moderate (2), or strong (3). Given that each stromal sample was represented in triplicate, the sum of the three values was calculated to obtain a score ranging from 0–9. This score was finally categorized into four groups to optimize the statistical analysis and to be expandable for the following: 0 (score 0–1), 1 (score 2–3), 2 (score 4–7), and 3 (score 8–9).

2.5. Statistical analysis

Differences in protein expression TS *versus* NFT were evaluated by chi-square test. Relationships between protein expression and clinical parameters were evaluated by chi-square or Fisher exact probability test for categorical