BASIC AND TRANSLATIONAL—LIVER

Prognostic Gene Expression Signature for Patients With Hepatitis C–Related Early-Stage Cirrhosis

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BACKGROUND & AIMS: Cirrhosis affects 1% to 2% of the world population and is the major risk factor for hepatocellular carcinoma (HCC). Hepatitis C cirrhosis–related HCC is the most rapidly increasing cause of cancer death in the United States. Noninvasive methods have been developed to identify patients with asymptomatic early-stage cirrhosis, increasing the burden of HCC surveillance, but biomarkers are needed to identify patients with cirrhosis who are most in need of surveillance. We investigated whether a liver-derived 186-gene signature previously associated with outcomes of patients with HCC is prognostic for patients with newly diagnosed cirrhosis but without HCC. **METHODS:** We performed gene expression profile analysis of formalin-fixed needle biopsy specimens from the livers of 216 patients with hepatitis C–related early-stage (Child–Pugh class A) cirrhosis who were prospectively followed up for a median of 10 years at an Italian center. We evaluated whether the 186-gene signature was associated with death, progression of cirrhosis, and development of HCC. **RESULTS:** Fifty-five (25%), 101 (47%), and 60 (28%) patients were classified as having poor-, intermediate-, and good-prognosis signatures, respectively. In multivariable Cox regression modeling, the poor-prognosis signature was significantly associated with death ($P = .004$), progression to advanced cirrhosis ($P < .001$), and development of HCC ($P = .009$). The 10-year rates of survival were 63%, 74%, and 85% and the annual incidence of HCC was 5.8%, 2.2%, and 1.5% for patients with poor-, intermediate-, and good-prognosis signatures, respectively. **CONCLUSIONS: A 186-gene signature used to predict outcomes of patients with HCC is also associated with outcomes of patients with hepatitis C–related early-stage cirrhosis. This signature might be used to identify patients with cirrhosis in most need of surveillance and strategies to prevent the development of HCC.**

Keywords: Liver Cancer Prevention; Early Detection; Screening; Whole Genome Gene Expression Profiling.

Cirrhosis represents the terminal stage of many chronic fibrotic liver diseases and is estimated to affect 1% to 2% of the world population.[1,2] Chronic infection with hepatitis C, afflicting 170 million individuals, is increasingly the cause of cirrhosis together with alcohol abuse in developed countries, and it superseded human immunodeficiency virus as a cause of death in the United States by 2007.[3] Cirrhosis-related mortality is high, with deaths attributable to cirrhosis-associated complications such as gastrointestinal bleeding or to hepatocellular carcinoma (HCC), which occurs in one-third of cirrhotic patients.[4] Even after complete surgical resection or local ablation of early HCC tumors, most patients develop subsequent de novo tumors due to a cancer-prone microenvironment in the cirrhotic liver referred to as the "field effect."[5]

With the development of noninvasive imaging and laboratory tests such as ultrasound-based liver stiffness measurement, cirrhosis has been increasingly diagnosed at an early stage and patients have been subjected to regular surveillance for HCC.[6] Clinical management of this growing patient population poses a challenge for cost-effective allocation of medical resources.[7] In addition, a number of chemopreventive strategies are being explored to abrogate the lethal complications of cirrhosis, which include hepatic decompensation and HCC.[1,2,8,9] Such interventions are often accompanied by significant toxicity and are

Abbreviation used in this paper: HCC, hepatocellular carcinoma.

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expensive.[10,11] Hence, biomarkers identifying patients at highest risk among those with early-stage cirrhosis would be extremely useful not only to enable cost-effective tumor surveillance but also to prioritize patients for chemopreventive intervention.[9]

Clinical staging systems such as the Child–Pugh classification[12] and Model for End-Stage Liver Disease score[13] are only able to distinguish advanced-stage from early-stage cirrhosis. In early-stage cirrhosis, only a handful of laboratory variables (eg, serum albumin level, bilirubin level, and platelet count) may carry prognostic information. However, their prognostic capability is limited because the measurement is generally within a normal range and its smaller dynamic range is more affected by nonspecific fluctuation.[1] Similarly, newer imaging- and laboratory test–based approaches have limited sensitivity for the detection of fibrotic progression and/or fibrogenic/carcinogenic activity in the cirrhotic liver.[2,14] Thus, there is a pressing need for robust and sensitive prognostic biomarkers for patients with early-stage cirrhosis.

We recently reported a 186-gene expression signature in liver tissue that was predictive of overall death in mostly hepatitis C–infected cirrhotic patients who had surgically treated primary HCC.[15] Molecular pathway analysis suggested that the signature reflects the field effect in cirrhotic liver. Therefore, in the present study, we hypothesized that the 186-gene signature might be a sensitive predictor of future risk of poor outcome in patients with newly diagnosed hepatitis C–related early-stage (Child–Pugh class A) cirrhosis who never developed HCC or any of the complications of cirrhosis at the time of diagnosis. Importantly, patients with such early-stage cirrhosis, which is far more prevalent than HCC, lack effective predictors of clinical outcome. To test our hypothesis, we evaluated the ability of the signature to predict clinical outcome from needle liver biopsy specimens obtained from an independent cohort of 216 patients with hepatitis C–related Child–Pugh class A cirrhosis who were prospectively followed up for a median of 10 years in the context of an HCC surveillance program[16–18] (prospective-retrospective design proposed to facilitate biomarker development[19]). We also evaluated the signature in multiple assay platforms to assess its clinical applicability.

Patients and Methods

Patients

Patients with a diagnosis of histologically confirmed cirrhosis lacking evidence and a history of hepatic decompensation or HCC were enrolled in the study between 1985 and 1998 and were prospectively followed up for the development of hepatic decompensation, HCC, or death as previously described ("Italian cirrhosis cohort for HCC surveillance").[16–18] Abdominal ultrasonography and esophagogastroduodenoscopy were performed before enrollment. Needle biopsy specimens of the liver were obtained within 2 years before enrollment and archived as formalin-fixed, paraffin-embedded tissue blocks. The patients received prospective follow-up every 6 months (see Supplementary Materials and Methods for details of patient enrollment and follow-up). Among the 360 enrolled cohort patients with various etiologies, including viral hepatitis and alcohol abuse, 216 patients with hepatitis C–related Child–Pugh class A cirrhosis were analyzed in this study (Figure 1). The study was approved by the review board of each participating institution on the condition that all samples were anonymized.

Gene Expression Profiling

Whole-genome gene expression profiling was performed using the complementary DNA–mediated Annealing, Selection, extension, and Ligation (DASL) DNA microarray assay (Illumina, San Diego, CA) as previously described[20] (see Supplementary Materials and Methods). Microarray data are available at National Center for Biotechnology Information Gene Expression Omnibus (GSE15654).

Statistical Analysis

Outcome prediction was performed using the 186-gene signature as previously described (see Supplementary Materials and Methods for details).[15,21] The log-rank test and Cox regression modeling were used to evaluate association of the signature and clinical variables with time from enrollment to overall death, liver-related death, occurrence of hepatic decompensation (gastrointestinal bleeding, ascites, or hepatic encephalopathy), progression of Child–Pugh class, and development of HCC. Liver-related death was defined as death due to liver failure and/or progression of HCC. Analyses were performed using either the GenePattern analysis toolkit[22] (www.broadinstitute.org/genepattern/) or the R statistical package (www.r-project.org).

Results

Needle Biopsy Expression Profiling

Because the standard approach to assessing cirrhosis in the clinical setting involves needle biopsies followed by formalin fixation, we first sought to assess the feasibility of performing genome-wide expression profiling on such small samples (typically 10 mm × 1 mm pieces of tissue). We previously showed that it is feasible to profile the expression of ~ 6000 transcripts in large formalin-fixed, paraffin-embedded specimens obtained from surgical resection. Here, we tested the ability of the assay to profile expression of all ~24,000 protein-coding genes in the human genome in fixed needle biopsy specimens. Of 280 patients with hepatitis C–related Child–Pugh class A cirrhosis, 236 patients with a sufficient amount of formalin-fixed, paraffin-embedded tissue blocks were subjected to gene expression profiling. Among them, 216 (92%) yielded high-quality genome-wide expression profiles (Supplementary Figure 1). The clinical demographics of the patients were not changed by the exclusion of poor quality profiles (Supplementary Table 1). Although not perfect, this result was remarkable because of (1) the tiny size of the specimens, (2) the age of the archived specimens (up to 23 years old), and (3) the fact that the samples were not collected with gene expression profiling as a primary goal.