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CYP1A2 is a predictor of HCC recurrence in HCV-related chronic liver disease: A retrospective multicentric validation study

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ABSTRACT

Background: Although hepatic resection is a potentially curative treatment for hepatocellular carcinoma (HCC), post-operative prognosis remains unsatisfactory due to the high incidence of recurrence. Several clinicopathological markers have been associated with HCC recurrence, but none has been validated. Extratumoral expression of cytochrome P4501A2 (CYP1A2) was recently proposed as predictor of HCC recurrence.
Aims: To validate extratumoral CYP1A2 as predictor of HCC recurrence and to determine its applicability to pretreatment liver biopsy.
Methods: Surgically resected HCC (n.180) with clinicopathological data and follow up were retrospectively studied (HCV n.54; HBV n.91; NAFLD/NASH n.35). CYP1A2 expression was evaluated using an immunohistochemical assay and semiquantitative analysis.
Results: Etiology-stratified analysis showed that low CYP1A2 expression was independently associated with recurrence-free survival in HCV patients (HR 2.814, 95% CI 1.300–6.093, p = 0.009); this association was lost in the whole cohort. Pretreatment liver biopsy and paired surgical specimens showed concordant CYP1A2 expression in the vast majority of cases (87%), with NPV of 100%, PPV of 81.25%, and a Cohen kappa of 0.72 (substantial agreement).
Conclusion: We validated the extratumoral expression of CYP1A2 as a biomarker of HCC recurrence in HCV patients. CYP1A2 analysis in pretreatment liver biopsy can be of help to stratify HCC patients for personalized treatment.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and a common cause of death in patients with chronic liver disease [1,2]. HCC management relies upon surgical, locoregional, and systemic treatment, selected according to the Barcelona Clinic Liver Cancer (BCLC) criteria [3]. The vast majority of patients experience early/late HCC relapse [4,5], emphasizing

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the need for reliable pretreatment predictors of outcome. Individual tissue markers exploring the phenotypic tumor profile and that of the surrounding background are expected to be of aid to further stratify patients before treatment.

It has been suggested that the extratumoral liver microenvironment plays an important role in HCC relapse [6]. A study has reported that the extent and pattern of fibrosis/cirrhosis can predict recurrence after resection [7], while another one has found this influence attenuated by virus-related factors [8]. Recently, a molecular pathway analyses suggested that a gene-expression signature in non-tumoral liver tissue can be used to predict HCC onset and outcome, reflecting a "field effect" of the surrounding liver on the natural history of HCC, particularly in HCV patients [9,10].

One molecular marker indicative of the non-tumoral liver metabolic background is cytochrome P_{450}1A2 (CYP1A2). This molecular indicator of hepatic oxidative stress, easily detectable in the non-cancerous areas of the liver, has been indicated as a valuable marker of fibrotic progression in HCV patients [11] and of pathological progression in nonalcoholic fatty liver disease [12]. More recently, Tanaka et al. using gene expression and immunohistochemical analysis [13] suggested that CYP1A2 expression is an independent predictor for postoperative recurrence of early-stage HCC.

The aims of the present study were to further investigate the expression of CYP1A2 in HCC patients with different etiologies of the background liver disease, to validate its role as predictor of HCC recurrence after resection, and to evaluate the suitability of the liver biopsy for this analysis.

2. Materials and methods

2.1. Study design

One hundred and eighty surgically resected HCC with clinicopathological information were retrospectively selected for this study. Of these, 54 were HCV-related, obtained from a European series (Milano, Italy; Bordeaux, France), 91 were HBV-related, obtained from an Asian series (Seoul, Korea) and 35 were NAFLD/NASH-related, obtained from an American series (Seattle, USA). Finally, a supplementary series of 23 cases with available pretreatment liver biopsy and paired surgical specimens, of different etiology and stage of disease, was used to evaluate the reproducibility of the immunohistochemical assay in the liver biopsy. The pre-operative diagnosis of HCC was based on dynamic imaging studies, biopsy, and alpha-fetoprotein serology (AFP) (data available only for European and Korean patients), according to EASL-EORTC/AASLD guidelines [14,15]. Before surgery, the size, number of lesions, and site of HCC were confirmed by imaging studies. The pre-operative HCC staging was determined using the BCLC staging system [16]. After resection, patients were followed up with ultrasonography (and serum AFP when available) every month, and with computed tomography and magnetic resonance imaging every three months, to determine recurrence and recurrence-free survival time (RFS).

2.2. Histologic evaluation of HCC

The three working groups (European, Asian, and American) independently performed the preliminary histologic evaluation of liver specimens. Surgical specimens were routinely processed and stained by hematoxylin and eosin. Histopathologic analyses were performed and the variables recorded for each case included tumor gross morphology, tumor size, differentiation according to the Edmondson–Steiner grade, presence of multiple tumors, microvascular invasion, stage of background liver disease according to the METAVIR classification, and margins status. In cases of multiple tumors, the one with the greatest diameter was selected for morphological analysis. Microvascular invasion was defined as groups of tumoral cells adhering to the endothelial lining of a vascular space at some point(s) (and not merely free-floating within the lumen), located in the non-tumoral liver usually in the proximity of the tumor margins or within the tumor capsule and only visible under the microscope.

2.3. CYP1A2 immunohistochemical evaluation

Immunohistochemical staining for CYP1A2 and evaluation were centralized in the Milan laboratory. According to the procedure and reagents used by Tanaka et al. [13], the anti-CYP1A2 antibody (3B8C1: sc 53614; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used at 1:500 dilution with phosphate-buffered saline containing 1% bovine serum albumin (Sigma–Aldrich, St. Louis, MO); the reaction was developed in an automated immunostainer (Ventana XT System; Ventana Medical System, INC., Tucson, AZ). Extratumoral CYP1A2 staining was then evaluated and semiquantitatively scored under light microscope by at least two independent investigators (MR, LDT). According to Tanaka et al. [13], cases showing 25–100% immunoreactive CYP1A2 extratumoral hepatocytes were considered high expressors and those with 0–24% extratumoral hepatocytes as low expressors. Staining intensity was not considered. Representative features of CYP1A2 assay results in surgical and biopsy specimens are shown in Fig. 1.

2.4. Statistical analysis

Data are expressed as number and percentage, or means and standard deviation (SD), or median (range), as appropriate. Variables were compared using the Fisher's exact test for categorical data and Kruskal Wallis rank test for continuous variables. The cumulative recurrence-free survival rates were calculated using the Kaplan–Meyer method. All possible prognostic factors were submitted to a Cox proportional-hazard regression. All factors with a p level <0.2 then entered in a multivariate Cox regression. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) are also indicated. A p-value <0.05 was considered significant. All tests were two-sided. All analyses were performed with Stata 13 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp. LP). The agreement of the two series was evaluated with Cohen kappa. In the estimation of agreement, a kappa coefficient value of 0.20 or less indicates slight agreement, a value between 0.21 and 0.40 reflects fair agreement, between 0.41 and 0.60 indicates moderate agreement, between 0.71 and 0.80 indicates substantial agreement, and a value of 0.81 or greater indicates almost perfect agreement.

3. Results

3.1. Baseline features of the series

Clinicopathological baseline features of the sample are shown in Table 1. The majority of patients were male (76.7%), with a mean age of 60.6 years (±10.9), and cirrhosis (57.2%). Table 1 also shows the sample characteristics divided by tumor etiology: HCV, HBV, and NAFLD/NASH. These three groups were significantly different in terms of many clinicopathological features, including age, gender, HCC features, and CYP1A2 expression. Most of the patients harbored a single HCC (139, 77.2%) with a mean size of 4.5 ± 3.5 cm. BCLC staging was almost equally distributed between stages A and B, with only 5 cases in stage C (2.8%), the latter NAFLD/NASH-related and most having a non-cirrhotic background. Most HCC had a moderate to poorly differentiated histology, while microscopic vascular

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