Immunoexpression of Heat Shock Protein 70, Glypican 3, Glutamine Synthetase, and Beta-Catenin in Hepatocellular Carcinoma After Liver Transplantation: Association Between Positive Glypican 3 and Beta-Catenin With the Presence of Larger Nodules

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ABSTRACT

Background. Hepatocellular carcinoma (HCC) is the 6th leading cause of cancer worldwide. Its recurrence ranges from 6% to 26%. In the literature, many factors are associated with higher risk of recurrence, without a clear definition of the best method that could predict this highly lethal event.

Objective. The aim of this study was to evaluate the immunoexpression of immunohistochemical markers: HSP70, glypican 3, glutamine synthetase, and beta-catenin, as well as studying their association with tumor characteristics and prognosis of patients undergoing liver transplantation for HCC.

Methods. We studied 90 patients who underwent liver transplantation from 1998 to 2012. Afterwards we evaluated factors related to survival, tumor recurrence, and the correlation of expression of the immunohistochemical markers.

Results. Immunohistochemical marker glutamine synthetase showed a positive trend toward better survival. HSP70-positive patients had a higher prevalence of histologic grade III. Patients with positive glypican 3 showed larger lesions and a higher number with AFP >200 ng/mL. Patients with positive beta-catenin showed larger nodules and more with histologic grade III. The association between beta-catenin and glypican 3 showed positive association with larger nodules.

Conclusions. Most of the markers studied had a correlation with at least one of the variables studied, confirming our hypothesis that these markers can indeed assist in assessing the prognosis of patients undergoing liver transplantation for HCC.

HEPATOCELLULAR carcinoma (HCC) is the 6th leading cause of cancer worldwide, accounting for >500,000 deaths annually [1]. It is the 3rd leading cause of death from neoplastic disease and the leading cause of death in cirrhotic patients [1]. Liver transplantation in recent years has proved to be a good therapeutic option in selected cases within preestablished criteria, with a 5-year survival of ~70%. It has been demonstrated as an approach of choice in cirrhotic patients diagnosed with HCC because as well as treating the cancer in evidence, it also treats the underlying disease [1–3]. Although survival rates are ~70% in 5 years, recurrence of HCC after surgery has risen, with rates ranging in the literature from 6% to 26% [3].

Recurrence of HCC after liver transplantation is often more aggressive than recurrence after liver resection,

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probably owing to the presence of immunosuppression used after transplantation [3]. There is no consensus therapy for the treatment of HCC recurrence after liver transplantation. The median survival from diagnosis of recurrence is only 12 months, so the prevention of tumor recurrence through a careful selection of patients to be transplanted is still considered to be the conduct of choice [3].

Many factors are associated with the higher risk of recurrence in the literature: lesion size, number of nodes, histological grade of the tumor, presence of macro or microvascular invasion, level of alpha-fetoprotein (AFP) above 200 ng/dL, immunosuppressive regimen used, among others [1–3]. There is no consensus about the best method to define the response that a HCC patient will have after liver transplantation [1–3].

Immunohistochemical studies have demonstrated increasing relevance in aggregating information about cellular features of malignancy [4]. The main markers used for this purpose are heat shock protein 70 (HPS70), glutamine synthetase, glypican 3 (GPC3), and beta-catenin.

HSP70 belongs to the class of proteins implicated in tumor genesis, which is the regulation of cell cycle progression and protection against spontaneous apoptosis generated by antineoplastic therapy [5]. GPC3 is a member of the heparan sulfate proteoglycan family and highly expressed in cells of lesions consistent with HCC [6]. Glutamine synthetase catalyzes the synthesis of glutamine in the liver of mammals. It is recognized as the largest source of energy used by tumor cells [7]. The signaling pathway of beta-catenin is essential in the process of development, differentiation, proliferation, growth, survival, cell regeneration, and remodeling [8].

The reason for these immunohistochemical analyses was their ability to infer specific cellular characteristics and therefore properties of malignancy of HCC lesions, assisting in the management of HCC liver transplant patients.

The aim of the present study was to evaluate the immunoexpression of these immunohistochemical markers, studying their association with tumor characteristics and prognosis of patients undergoing liver transplantation for HCC.

METHODS

This study was a longitudinal cohort, with the use of a database collected prospectively from 1996 to 2010 observing patients undergoing liver transplantation with evidence of HCC in explants.

The immunohistochemical study was performed experimentally and prospectively from 2011 to 2012 with the use of tissue samples collected from explants stored by the Department of Pathology, Hospital de Clinicas (HC), Faculty of Medical Sciences, State University of Campinas.

Cases were included regardless of race or sex as long as they were adults (>18 years of age) and had undergone a liver transplantation by the piggyback or conventional technique. All of the patients were diagnosed individually with HCC by the same pathologist at the HC Liver Transplant Unit. Cases were discarded when it was not possible to perform one of the coloring techniques (hematoxylin-eosin or immunohistochemical study) or the tumor architecture could not be recognized.

All patients received a liver graft from cadaver donors according to Brazilian law. General variables were collected from the receiver: name, age, sex, etiology of chronic liver disease, Model for End-Stage Liver Disease (MELD) score immediate before surgery, and AFP levels. In the study of the explant, the following topics were evaluated: histologic grade of the tumor according to Edmondson-Steiner classification, size of the largest nodule, number of nodules, and presence or absence of microvascular invasion.

Evaluation of the explants indicated the presence of HCC in 120 patients. However, owing to the loss of archival material and in some cases the absence of viable tumors studied in the blocks, the final number of samples obtained was decreased to 90 cases.

Tissue microarray (TMA) was the method used in the manufacture of blades for the study. The protocol used in the immunohistochemical technique involved the following steps: deparaffinization, blocking endogenous peroxidase, antigen retrieval, and primary antibody staining. The antibodies used were for HSP70, GPC3, glutamine synthetase, and beta-catenin. Following established convention and to enhance the statistical investigation, markers were considered to be positive when they were present in >25% of the specimens in HCC cell sample cylinders.

To compare proportions, the chi-square test or Fisher exact test was used. For comparison of numeric measurements between 2 groups, the Mann-Whitney test was used. To identify factors associated with death, univariate and multiple logistic regressions were performed. To identify factors associated with survival, univariate and multivariate Cox regressions were applied. The actuarial survival analysis was carried out with the use of Kaplan-Meier and Breslow tests. For the process of selection of variables, a stepwise procedure was used. To determine the cutoff values, we used the receiver operating characteristic. The significance level for statistical tests was 5%. The software used was SAS System for Windows (Statistical Analysis System), version 9.2 (SAS Institute, Cary, North Carolina).

Informed consent was obtained from each patient. The Ethics Committee of the Medicine Faculty of our institution gave the approval for this research, and the reported investigations were carried out in accordance with the principles of the Helsinki Declaration of 1975 as revised in 2008.

RESULTS

We evaluated 90 cirrhotic patients undergoing liver transplantation who had diagnostic confirmation of HCC. Among these 90 patients, 74 (82%) were male and 16 (18%) female. The overall average age was 52.8 ± 9.1 years. The MELD score, without extra points, observed at the time of surgery was 15 ± 5. Mean AFP was 111.38 ± 373.93 ng/mL.

The survival rate and the categoric variables sex, presence of recurrence, histology grade, presence of vascular invasion, and AFP >200 ng/dL were observed in patients with tumor recurrence who presented lower survival ($P = .04$; chi-square/Fisher test).

In the study of the relationship between survival and continuous variables age, number of nodules, size of the largest nodule, level of AFP, duration of surgery, and MELD scores, it was observed that patients with longer