Co-expression patterns of Notch1, Snail, and p53 in grade III hepatocellular carcinoma with postoperative recurrence: a preliminary study

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Background/Aims: We aimed to determine the association between the co-expression patterns of Notch1, Snail, and p53 proteins (NSP) and the postoperative prognosis of hepatocellular carcinoma (HCC). Methods: The immunoblot data for molecular expression (147 HCC/corresponding non-HCC tissues and 15 dysplastic nodules) and the sequencing data for p53 mutations (110 HCCs) were obtained from our previous study. Data analyses were restricted to cases with HCC differentiation grade III (n=47), due to its high p53 mutation rate. Results: Nineteen of the 47 patients (40.4%) comprising 12 in the liver and 7 in distant organs, had relapsed at 1-2 years after surgery. There was no relationship between p53 mutation and postoperative recurrence in the grade III HCCs. Seven (87.5%) of the eight relapsed cases with Notch1, Snail, and p53 (wild) co-expression experienced recurrence only within the liver, and all tumors were smaller than 5 cm in diameter. Extrahepatic relapse occurred mostly in HCC patients with tumors larger than 5 cm in diameter, without any deviation in the NSP pattern. Conclusions: The results of this preliminary study suggest that the co-expression of Notch1, Snail, and p53 (wild) is not inferior to the patterns with p53 mutation as an indicator of postoperative recurrence of grade III HCC. (Korean J Hepatol 2012;18:63-74)

Keywords: Notch1 intracellular domain; p53; Snail; p53 mutation; Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most common malignant tumors worldwide,¹ is usually treated by surgical resection, particularly in patients with American Joint Committee on Cancer (AJCC)-TNM stages I or II and reserved liver function.² Because postoperative recurrence is the main obstacle to the success of this aggressive treatment,³,⁴ various pathological features of surgical specimens have been established as predictors of risk of recurrence. They include tumor size and number, microvascular invasion, portal vein tumor thrombosis, the presence of satellite nodules, differentiation grade of the tumor cells, and the encapsulation state.²,⁵,⁶ Tumor size (usually greater than 5...
cm), multiplicity, a cut margin narrower than 1 cm, and tumor stage (including vascular invasion) are independent factors associated with early (within 2 years) recurrence after surgical resection. The preoperative hepatic functional reserve and microscopic vascular invasion can enhance early recurrence of small HCCs. Early multinodular recurrence after surgical resection is not unusual in HCCs with multiple tumor nodules and portal vein thrombi.

Furthermore, late recurrence (>2 years after resection) is significantly associated with Ishak hepatic inflammatory activity >6; indocyanine green (ICG) retention rate at 15 min (ICG-15) >10%; and hepatitis B virus (HBV) DNA level >10^6 copies/mL. Alpha-fetoprotein (AFP) and Lens culinaris agglutinin-reactive AFP (AFP-L3) are serological biomarkers of HCC with a more malignant nature. Multiple intrahepatic or extrahepatic metastases are associated with poor prognosis after recurrence. Reportedly, the proportions of intra- and extrahepatic recurrences of HCC following surgery are 80% and 20%, respectively. In brief, the organs involved in extrahepatic recurrence are lung (10.4-12.6%), adrenal glands (11%), peritoneum (6%), pancreas (3%), bone (1.5%), and brain (1.5%).

Given the increasing chances of earlier diagnosis by an HCC screening program for high-risk individuals selected from patients with chronic liver diseases, it is critical to identify the characteristic patterns of molecules that are associated with HCC recurrence and the preferred organ sites of relapse after surgical treatment. Candidate molecules associated with prognosis or risk of recurrence include osteopontin and stathmin in relation with intrahepatic recurrence of HCCs, the acquisition of p53 mutation (p53Mut) as a poor prognostic molecular marker, and loss of E-cadherin with causal relationship to the invasiveness of HCC. In our previous study, we have suggested that an orchestration of multiple molecules engage in the regulation of malignant cancer cell behaviors such as invasion and proliferation. Further, we arbitrarily defined the co-expression patterns of Notch1, Snail, and p53 (NSP) proteins based on immunoblot analysis of clinical samples and matched them with experimentally observed malignant behavior. We proved that the expression status of the wild type of p53 (p53WT)/p53Mut interactively regulated Notch1 expression, and simultaneous upregulation of p53WT together with Notch1 and Snail expressions represented the invasive nature of HCCs. In this study, we verified the clinical utility of the NSP pattern as postoperative prognostic molecular markers for HCC. Because most of the p53 mutations occurred in grade III HCCs, data analyses were restricted to grade III HCCs to compare the effects of NSP patterns on the prognosis of HCC patients with p53WT and p53Mut.

MATERIALS AND METHODS

Inclusion of patients with immunoblot data for Notch1, Snail, and p53 and data for p53 mutations in HCCs

For this study, we obtained 162 immunoblots for the expression of Notch1, Snail, and p53 proteins and 110 sequences for p53 mutations in HCCs. All experiments were performed as described in the previous study. Briefly, rabbit monoclonal anti-Notch1 (diluted 1:100, Cell Signaling, Danvers, MA), rabbit polyclonal anti-Snail (diluted 1:100, Abcam, Cambridge, MA), and mouse monoclonal anti-p53 (diluted 1:100, Santa Cruz Biotechnology) antibodies were used to detect each protein in paraffin sections, and automated sequencing was performed to detect p53 mutations in frozen tissues (Appendix Fig. 1). Of the 162 immunoblots obtained, 147 were from patients with HCC (33 grade I, 40 grade II, and 74 grade III); their corresponding non-HCCs and 15 dysplastic nodules (DN) were used as controls. All tissue specimens, along with clinicopathological information, were obtained from Severance Hospital, Yonsei University College of Medicine (Seoul, Korea). The analyses focused on the effects of differentiation grade III HCC on postoperative prognosis, for which 47 cases were chosen on the basis of well-defined clinical records (mean age, 53±13 years; range, 28-76 years; 38 men and 9 women). Together with biochemical liver function, HBV viral markers, and serum AFP and protein induced by vitamin K absence (PIVKA) tests, all patients underwent a liver spiral computed tomography (CT) scan every 3-6 months to detect recurrent HCC after surgery. All patients had HBV infection and reserved liver function (Child-Pugh A). The reason for this selection criterion was that the differentiation grade and poor liver function themselves influenced the postoperative prognosis, and etiological factors could influence molecular expression patterns.