

Smad3 and its phosphoisoforms are prognostic predictors of hepatocellular carcinoma after curative hepatectomy

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BACKGROUND: Progression of hepatocellular carcinoma (HCC) often leads to vascular invasion and intrahepatic metastasis, which correlate with recurrence after surgical treatment and poor prognosis. HCC may be an unusual cancer affected by continuous inflammation that can lead to consistent upregulation of transforming growth factor- β (TGF- β). Chronic inflammation shifts hepatocytic TGF- β signaling from the tumor-suppressive pSmad3C pathway to the oncogenic pSmad3L pathway. In this study, we investigated the functional roles of Smad3 and its phosphoisoforms in the progression of HCC.

METHODS: Tumor tissue microarrays of samples from 272 HCC patients who underwent curative surgical resection were used to detect the expression of Smad3, Smad4, pSmad3C (S423/425), pSmad3L (T179), pSmad3L (S204), and pSmad3L (S213). Disease-specific death was defined as 1) tumor occupying more than 80% of the liver, 2) portal venous tumor thrombus (PVTT) proximal to the second bifurcation, 3) obstructive jaundice due to tumor, 4) distant metastases, or 5) variceal hemorrhage with PVTT proximal to the first bifurcation. At the time of analysis, tumor recurrence was detected in 184 (67.6%) patients, and 96 (35.3%) had died of HCC.

RESULTS: Nuclear and cytoplasmic localization of Smad3, and nuclear localization of Smad4 were observed in 18.0%, 9.9%, and 9.2% of HCCs, respectively. The rates of Smad3 phosphoisoform-immunoreactive HCC varied according to the location of phosphorylation: pSmad3C (S423/425) 8.1%, pSmad3L (T179) 2.6%, pSmad3L (S204) 2.2%, and pSmad3L (S213) 10.3%. Multivariate analyses revealed that pSmad3C (S423/425) ($P=0.022$) was an independent predictor of longer recurrence-free survival. pSmad3L (S213) ($P=0.006$),

intrahepatic metastasis, multicentric occurrence, and liver cirrhosis were independent predictors of shorter recurrence-free survival. Cytoplasmic Smad3 ($P=0.006$), larger tumor size, and intrahepatic metastasis were independent predictors of shorter disease-specific survival. Only pSmad3L (S213) did not show an unfavorable influence on recurrence-free survival ($P=0.331$) on univariate analysis.

CONCLUSIONS: pSmad3C (S423/425), pSmad3L (S213), and Smad3 may be predictors of prognosis in HCC patients after curative hepatectomy. pSmad3C (S423/425) and pSmad3L (S213) may be used as immunohistochemical biomarkers to identify patients with a high risk of recurrence.

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KEY WORDS: Smad3;
pSmad3C (S423/425);
pSmad3L (S213);
hepatocellular carcinoma;
hepatectomy;
survival

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, especially in Asia and Africa.^[1] With continued surveillance and advances in imaging, the detection rate of localized HCC has increased, resulting in an increase in the curative surgical resection rate. However, the prognosis of HCC remains poor because of a high rate of recurrence or metastases after surgery. Cancer classification using biomarkers can identify patients with a high risk of recurrence or metastases.^[2] Further investigation of these biomarkers would provide personalized therapy according to the predicted risk of recurrence.

The transforming growth factor- β (TGF- β) signaling pathway is known to play an important role in cellular development, differentiation, proliferation, migration, and neoplasia.^[3] TGF- β maintains tissue homeostasis and prevents incipient tumors from progressing to

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malignancy. However, misregulation of TGF- β signaling promotes tumor growth and invasion, evasion of immune surveillance, and cancer cell dissemination and metastases.^[4] The binding of TGF- β to the TGF- β type II receptor induces phosphorylation and activation of the TGF- β type I receptor (T β RI). The activated T β RI triggers activation of Smad3 by phosphorylation at the C-terminal serine residues (S423/425), which forces Smad3 to dissociate from its membrane-bound receptors and form a heteromeric complex with Smad4. The phosphorylated Smad3 accumulates in the nucleus and either activates or represses the transcription of a selected set of target genes.^[5] Smad3 contains a conserved N-terminal (Mad-homology 1) domain that binds DNA, and a conserved C-terminal (Mad-homology 2) domain that interacts with T β RI, other Smad proteins, and various transcriptional coactivators/corepressors.^[4] These two highly conserved domains are separated by a less-conserved linker region which contains four proline-directed kinase phosphorylation sites: Thr179, Ser204, Ser208 and Ser213. Phosphorylation at these sites is performed by Ras-associated kinases, including extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK), and cyclin-dependent kinase 4 (CDK4), and is known to promote carcinogenesis. Conversely, phosphorylation at the C-terminus (Ser423/425) is performed by T β RI and the activin type I receptor, and is reported to be tumor-suppressive. Linker phosphorylation of Smad3 indirectly inhibits C-terminal phosphorylation and subsequently suppresses tumor-suppressive pSmad3C signaling.^[6,7]

JNK-dependent pSmad3L accelerates cancer development in the human colorectum^[8] and Smad3 phosphorylated at both the linker and COOH-terminal regions transmits a malignant TGF- β signal in later stages of human colorectal cancer.^[9] T β RI-dependent pSmad3C transmits a tumor-suppressive TGF- β signal, while JNK-dependent pSmad3L promotes carcinogenesis in human chronic liver diseases.^[10] It has been reported that pSmad3L/c-Myc is enhanced in rat hepatocytes exposed to diethylnitrosamine (DEN), and pSmad3C/p21WAF1 is impaired as DEN-induced rat HCC develops and progresses.^[11] The rat model of DEN-induced HCC has a histology and genetic signature similar to that of human HCC with poor prognosis.^[12] Recent studies showed that low pSmad3C and high pSmad3L positivity are significantly predictive of human HCC development within 12 years.^[13,14] However, Smad3 phosphoisoforms in HCC have been controversial and their role in HCC progression remains unclear. In this study, we investigated the roles of Smad3, Smad4, pSmad3C (S423/425), pSmad3L (T179), pSmad3L (S204), and pSmad3L (S213)

in HCC progression in samples from 272 HCC patients with long-term follow-up using tissue microarrays.

Methods

Patients and histopathology

A total of 272 consecutive primary HCC samples were collected from patients who underwent hepatectomy at the Samsung Medical Center (Seoul, Korea) from July 2000 to May 2006. Patient ages ranged from 21 to 76 years with an average of 52.5. The male-to-female ratio was 223:49. All patients were treated with curative surgical resection which, in most cases, was followed by second-line treatments at the time of recurrence. We defined curative resection as complete resection of all tumor nodules with clear microscopic resection margins and no residual tumors as indicated by computed tomography one month after surgery. None of the patients received preoperative chemotherapy. This study was approved by the Institutional Review Board of Samsung Medical Center.

Clinical parameters, including age, gender, date of surgery, and tumor size were obtained from pathology reports. The histopathological features of HCCs examined by two pathologists (Kim SH and Park CK) were histological differentiation, microvascular invasion, major portal vein invasion, intrahepatic metastasis, multicentric occurrence, and non-tumor liver pathology. HCCs were graded histologically according to the criteria of Edmondson and Steiner.^[15] Microvascular invasion was considered present when one or more endothelial cells or the tunica media of the vessel surrounded a neoplastic cell group. Intrahepatic metastasis and multicentric occurrence were matched to the criteria of the Liver Cancer Study Group of Japan.^[16]

Patient serum levels of α -fetoprotein and computed tomography were performed at least once every 3 months after surgery until December 31, 2010. When tumor recurrence was suspected, precise diagnostic imaging was performed by magnetic resonance imaging. Recurrence-free survival (RFS) was defined from the date of resection until the detection of tumor recurrence. While HCC is the cause of death in most patients with the disease, some patients die of liver failure or other causes in the absence of progressive HCC (24 of the 120 deaths in this study were of non-HCC causes). We chose HCC-related mortality (disease-specific death) as the clinical endpoint for survival analysis, defined as follows: 1) tumor occupying more than 80% of the liver, 2) portal venous tumor thrombus (PVT) proximal to the second bifurcation, 3) obstructive jaundice due to

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