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Role of Sox2 and Oct4 in predicting survival of hepatocellular carcinoma patients after hepatectomy

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ABSTRACT

Objectives: The present study was aimed to explore the prognostic strength of Sox2 and Oct4A in hepatocellular carcinoma (HCC).

Design and methods: We investigated the expression of Sox2 and Oct4A in five hepatoma cell lines, one immortalized normal liver cell line, HCC tissues with matched nontumorous liver tissues and normal liver tissues by reverse transcription-polymerase chain reaction.

Results: Sox2 and Oct4A mRNA were overexpressed in hepatoma cell lines and tumor tissues. Sox2 or Oct4A positive expression was significantly associated with an aggressive phenotype. Both univariate and multivariate analyses revealed that Sox2 or Oct4A was an independent prognostic factor for HCC. When using subgroup analysis, the patients with a co-expression of Sox2/Oct4A had the poorest prognosis. Further analysis demonstrated that Sox2 alone or Sox2/Oct4A could stratify outcome in HCC patients with early stage.

Conclusions: Sox2 and Oct4A can be novel predictors of poor prognosis for patients undergoing resection of HCC.

Introduction

Hepatocellular carcinoma (HCC), one of the most devastating diseases in the world, comprises more than 90% of human liver cancers, and about 55% of all HCC incidences are identified in China. Despite the recent advances in early detection and patient management, the prognosis remains unsatisfactory, mainly because of a high postoperative recurrence and metastasis. At present, prediction for recurrence and prognosis for HCC patients are generally based on clinical staging systems. However, individuals with HCC in the same clinical stage have a highly variable outcome [1], which supports the notion that HCC comprises several biologically distinct subgroups. To provide the most qualified therapy for each HCC patient, it is required to classify HCC according to its unique biological nature. Thus intense efforts have been made to obtain appropriate biomarkers for identifying HCC patients with distinct clinical outcome [2].

It has been demonstrated that embryonic stem (ES) cell-associated transcription regulators play an important role in carcinogenesis, which raises the possibility that molecular characteristics of ES cells could provide supplementary information to identify distinct subgroup of cancers. Sox2 (sex-determining region Y-box 2), together with Oct4 (octamer binding transcription factor 4) plays a pivotal role in the maintenance of self-renewal and pluripotency of ES cells [3]. Sox2 is a critical transcription regulator for sustaining proliferation and tumorigenicity of glioblastoma tumor initiating cells [4]. It has been shown that Sox2 is expressed in a variety of solid tumors, including glioblastoma, breast cancer, pancreatic carcinoma, lung and esophageal squamous cell carcinomas and may act as an oncogene in these tumors [5–9]. However, overexpression of Sox2 in gastric cancer cells inhibits cell growth through cell-cycle arrest and apoptosis [10]. Therefore, Sox2 may behave differently among different cancers. Oct4 is a POU domain transcription factor expressed in ES cells and germ cells [11]. The human Oct4 gene encodes three isoforms, termed Oct4A, Oct4B and Oct4B1 [12]. Oct4A, the isoform mentioned in most reports, is known to be a partner of Sox2 and the pair activates downstream genes essential for ES cells [3]. It has been reported that knockdown of Oct4 reduces the clone formation and migration of Hep-G2 cells [13]. Our preliminary study which was reported in

Abbreviations: HCC, Hepatocellular carcinoma; RT-PCR, Reverse transcriptase-polymerase chain reaction; Sox2, Sex-determining region Y-box 2; Oct4, Octamer binding transcription factor 4; ES, Embryonic stem; AFP, alpha-fetoprotein; TNM, Tumor–node–metastasis; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B virus; OS, Overall survival; DFS, Disease-free survival.

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Chinese demonstrated that Oct4A mRNA was frequently overexpressed in HCC tissues, and correlated with a malignant phenotype [14]. In terms of the prognostic significance, a high level of Sox2 and Oct4 was found to be correlated with poor prognosis in human esophageal squamous cell carcinoma [15]. Expression of Sox2 or Oct4 could predict poor prognosis in rectal cancer patients who accept preoperative chemoradiotherapy [16]. Our previous data showed that the HCC patients with Oct4A positive expression tended to have poor prognosis [14]. Based on previous observations, we hypothesize that Sox2 might play a role synergistically with Oct4 in HCC development. In the present study, our intention was to evaluate the expression Sox2 and Oct4A in HCC by RT-PCR, and explore the predictive values of Sox2 and Oct4A for the prognosis of HCC patients.

Materials and methods

Cell lines

The human cell lines L-02 (an immortalized normal human liver cell line), MHCC-97L and MHCC-97H (hepatoma cell lines with low and high metastatic properties, respectively), and other hepatoma cell lines SMMC-7721, BEL-7402 and Hep-G2 were cultured by continuous passage in RPMI-1640 or DMEM supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin. Cells were maintained in a humidified incubator at 37 °C in 5% CO_2. RNA was extracted from exponentially growing cells.

Clinical specimens

Hepatic tissues were obtained from patients who underwent hepatectomy between 2001 and 2007 in a single group at the Department of Hepatobiliary Oncology, Sun Yat-sen University Cancer Center. The fresh tissues were frozen in liquid nitrogen immediately after resection and archived in the institution's liver tumor bank and stored at −80 °C until processing. Gene expression profiles were conducted in primary HCC tissues and matched adjacent nontumorous liver tissues from 136 consecutive patients with HCC who hadn't received preoperative transhepatic arterial chemembolization, radiotherapy or chemotherapy. Twenty normal liver tissues were obtained from the patients with hemangioma or focal nodular hyperplasia (FNH). The clinicopathologic variables were shown in Table 1. The diagnosis was confirmed histologically in all cases, based on detailed examination of sections stained with hematoxylin and eosin (H&E). All histologic assessments were made by an experienced pathologist. Written informed consent was obtained from all of the patients, and the study was approved by Clinical Research Ethics Committee of Sun Yat-sen University Cancer Center. Tumor staging were determined according to the 7th edition tumor–node–metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC).

RNA extraction and cDNA synthesis

Total RNA was extracted from cell lines and liver tissues using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. To remove any DNA contamination, RNA was treated with RNase-free DNase I (Invitrogen, Carlsbad, CA) and qualified by spectrophotometry and the concentration of RNA was measured by optical density at 260 nm. The first strand of cDNA was synthesized using oligo(dT)15 primer (Promega, Madison, WI), M-MLV Reverse Transcriptase (Promega, Madison, WI), and 2 μg of total RNA according to the manufacturer's instructions. For each sample, a no-reverse transcription (No-RT) control was used in parallel from the DNase-treated RNA to rule out any potential nonspecific amplification resulting from contaminated genomic DNA.

Table 1
Correlation between Sox2 or Oct4A mRNA expression and clinicopathalogic variables in 136 patients with HCC.

Variables	Cases	Sox2 mRNA		*P* value[a]	Oct4A mRNA		*P* value[a]
		Negative (n = 75)	Positive (n = 61)		Negative (n = 44)	Positive (n = 92)	
Gender							
Female	14	11	3	0.063	6	8	0.380
Male	122	64	58		38	84	
Age (years)							
≤50[b]	68	38	30	0.863	20	48	0.463
>50	68	37	31		24	44	
HBsAg							
Negative	15	6	9	0.211	4	11	0.773
Positive	121	69	52		40	81	
AFP (μg/L)							
≤400	85	50	35	0.266	25	60	0.344
>400	51	25	26		19	32	
Cirrhosis							
No	15	7	8	0.652	6	9	0.200
Mild	51	31	20		16	35	
Moderate	55	28	27		14	41	
Severe	15	9	6		8	7	
Child–Pugh							
A	118	65	53	0.970	39	79	0.656
B	18	10	8		5	13	
Tumor size (cm)							
≤5	51	34	17	0.036	23	28	0.014
>5	85	41	44		21	64	
Tumor number							
Single	93	54	39	0.314	34	59	0.123
Multiple	43	21	22		10	33	
Tumor encapsulation							
Complete	32	21	11	0.173	11	21	0.780
None	104	54	50		33	71	
Vascular invasion							
No	103	66	37	<0.001	40	63	0.004
Yes	33	9	24		4	29	
Differentiation							
I–II	76	50	26	0.005	27	49	0.373
III–IV	60	25	35		17	43	
TNM stage							
I	73	50	23	0.001	33	40	0.001
II–III	63	25	38		11	52	

Abbreviations: HCC—hepatocellular carcinoma, HBsAg—hepatitis B surface antigen, AFP—alpha-fetoprotein, TNM—tumor–node–metastasis.
[a] Chi-square or Fisher's exact test.
[b] Value is median.

Semiquantitative RT-PCR

A blinded semiquantitative RT-PCR analysis was carried out; no clinicopathologic or follow-up data were revealed to the bench researchers until the RT-PCR results were finalized. The primers and conditions used for Sox2, Oct4A and house keeping gene β_2-microglobulin (β_2m) were listed in Supplementary Table 1. The PCR products were analyzed on 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet (UV) illumination. All experiments were done in duplicate.

Follow-up

Follow-up was finished on March 26, 2009. The median follow-up time was 20 months (range, 1–83 months). All patients were monitored prospectively by physical examination, serum alpha-fetoprotein (AFP), abdomen ultrasonography, and chest X-ray every 1–3 months in the first year and every 3–6 months thereafter for surveillance of recurrence or metastases. For patients with test results suggestive of recurrence, computed tomography and/or magnetic resonance imaging and/or positron emission tomography were used to verify whether recurrence had occurred. Intrahepatic tumor recurrence or distant metastasis detected only by imaging diagnosis after tumor resection was designated as recurrence. During the