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Research paper

SOX1 is correlated to stemness state regulator SALL4 through progression and invasiveness of esophageal squamous cell carcinoma

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ABSTRACT

SOX1, as a tumor suppressor, play anti-tumorigenecity role in different cells and its expression is inhibited in a variety of cancers. The aim of this study was to evaluate SOX1 expression and its correlation with cancer stem cell (CSC) markers in ESCC. Using real time PCR, the relative comparative expression of SOX1 in 40 ESCC samples was assessed compared to related margin normal tissues, and its correlation with CSC markers including SALL4, SOX2, and MEIS1 was analyzed statistically. The results revealed significant under-expression of SOX1 in ESCC in significant correlation with different indices of poor prognosis including depth of tumor invasion ($P = 0.02$), Stage of tumor cell progression ($P = 0.05$), and number of involved lymph node metastasis ($P = 0.05$). Furthermore, the under-expression of SOX1 was associated significantly with SALL4 overexpression. This study was the first to evaluate SOX1 underexpression and its association with poor prognosis in ESCC. Since correlation of SOX1 and SALL4 was detected in advanced stages of ESCC progression, as well as high invasive and aggressive tumor tissues, it may be extrapolated that SOX1 expression may have critical role in inhibition of ESCC invasiveness and aggressiveness especially in advanced stages of the disease.

1. Introduction

Stem cell gene expression regulatory network is crucial to understand the molecular mechanisms governing both embryonic and cancer development (Kanterakis et al., 2008). Although, these networks have been studied and depicted (Kanehisa and Goto, 2000), deregulation of contributed transcription factors (TFs) in these networks may be involved in tumor progression and cancer stem cells (CSCs) biology.

Esophageal squamous cell carcinoma (ESCC), as the eighth most common cause of cancer-related death worldwide, is an aggressive disease with a low 5-year survival rate (Forghanifard et al., 2015; Kang et al., 2015). Since ESCC is asymptomatic in early stages, it is usually diagnosed at advanced stages of the disease, and current therapeutic modalities must be improved to increase patients' survival rate (Forghanifard et al., 2014a). Elucidation of stemness TFs in ESCC may help to understand the programmed network of gene expression, leading to introduce potentially new therapeutic targets (Forghanifard et al., 2014b). Deregulation of such TFs in ESCC were reviewed previously (Islam et al., 2015); however; expression pattern of SOX1 and its clinicopathological relevance in ESCC as well as its correlation with other stemness TFs was not studied yet.

SOX (sex determining region Y [SRY] related high-mobility group box) genes are belonged to SRY box genes superfamily. This group of genes are expressed in different human tissues having impact on activation of a variety of cell signaling pathways. Sox genes are categorized to 8 subtypes named from A to H (Kiefer, 2007). SOX1 belongs to subgroup B and play specific role in neural cell fate decision and differentiation (Archer et al., 2011).

SALL4, as C2H2 zinc-finger transcription factor, has main role in normal development, as well as stemness and self-renewal of embryonic stem cell. Overexpression of SALL4 has been reported in several cancers and recent meta-analysis elucidated its oncogenic role in solid malignancies in inverse correlation with disease-free survival (Cheng et al., 2016). Our recent data showed overexpression of SALL4 in ESCC (Forghanifard et al., 2014b). Nevertheless, its crosstalk with other transcription factor in regulatory network of CSC gene expression demand more investigation in the disease.

To elucidate expression pattern of SOX1 in ESCC and its correlation with other CSC markers such as SALL4, SOX2, and MEIS1, we aimed to study the clinicopathological relevance of SOX1 in ESCC patients.

Abbreviations: ESCC, esophageal squamous cell carcinoma; CSCs, cancer stem cells; TF, transcription factor; SOX, sex determining region Y [SRY] related high-mobility group box.

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Table 1
Primer sequences used for qRT-PCR.

Gene	Forward primer	Reverse primer
GAPDH	GGAAGGTGAAGGTCGGAGTCA	GTCATTGATGGCAACAATATCCACT
SOX1	TGAACGCCTTCATGGTGTGGTC	ATTACAAGTACCGGCCGCGC
SALL4	CCAAAGGCAACTTAAAGGTTCAC	GAGATCTCATTGGTCTTCACGG
MEIS1	ATGACACGGCATCTACTCGTTC	TGTCCAAGCCATCACCTTGCT
SOX2	AGCTACAGCATGATGCAGGA	GGTCATGGAGTTGTACTGCA

2. Materials and methods

2.1. Study population

Tumors and related margin non-tumor samples were collected from 40 ESCC patients who refer for esophagectomy to Omid Oncology Hospital at Mashhad University of Medical Sciences (MUMS), Mashhad, Iran. All patients were checked for neither preoperative chemo- nor radio-therapy experiences. The tumor and normal esophagus tissues were histologically analyzed. The demographic characteristics distinct based on the seventh edition of Union International Cancer TNM classification guidelines (Sobin et al., 2011). The study was performed after confirmation of Ethics Committee of the MUMS and declaration consents by all recruited patients.

2.2. RNA extraction, cDNA synthesis and qRT-PCR

Using TriPure RNA extraction reagent (Roche, Nutley, NJ), RNA was extracted from all tissues. The quality of RNA was assessed by gel electrophoresis. PrimeScript First Strand cDNA Synthesis Kit (Takara, japan) was used to synthesize cDNA. To evaluate gene expression, cDNA was amplified with specific primers (Table 1) using SYBR green method in Stratagene Mx-3000P real-time thermos-cycler (Stratagene, La Jolla, CA). ROX was used as reference dye. The following optimal thermal condition was applied: 10 min at 95 °C, 35 cycles of 15 s at 95 °C, 30 s at 57 °C, and 45 s at 72 °C. The real time PCR data were normalized by GAPDH and ΔΔCT method was used for fold change evaluation of gene expression in ESCCs compared to normal tissues (Raeisossadati et al., 2014). The tests were performed in triplicate.

2.3. Statistical analysis

Statistical analyses were carried out using the SPSS version 22 statistical package (SPSS, Chicago, IL). To correlate gene expression with demographic clinical features, the χ^2 or Fisher exac *t*-test, independent-sample *t*-test and ANOVA were mainly used. Pearson's correlation was used to analyze association between SOX1 and other genes expression. $P < 0.05$ was considered statistically significant.

Table 2
Correlation of SOX1 mRNA expression with clinicopathological features of ESCC patients.

Features	SOX1		P value
	Underexpression	Unchanged	
Sex			
Female	5	15	0.327
Male	4	16	
Node metastasis			
No metastasis	5	19	**0.045**[a]
Node metastasis	4	12	
Tumor invasion			
T1,2	1	7	**0.020**[a]
T3,4	8	24	
Surgical stage			
Stage I/II	5	21	**0.035**[a]
Stage III/IV	4	10	
Grade of differentiation			
P.D[b]	0	3	
M.D	6	23	0.752
W.D	3	5	
Location			
Lower	6	13	0.635
Middle	3	18	
Upper	0	0	

(Independent-Sample *t*-Test was used to analyze correlation between gene expression and sex, node metastasis, tumor invasion, and surgical stage. One-Way ANOVA test was used to correlate gene expression and tumor grade, and location).
[a] Significant correlation.
[b] PD: poorly differentiated, MD: moderately differentiated, WD: well differentiated.

3. Results

3.1. Study population and clinical demographic data

The frequency of female and male was equal. The mean age ± standard deviation (SD) of the enrolled patients was 62.17 ± 13.01. The size of tumor samples were ranged from 1.5 to 8 cm (mean ± SD: 3.9 ± 1.57), resected from middle or lower parts of esophagus. The tumor and normal tissues of each patient were analyzed histologically to confirm their characteristics. Using Haematoxilin and Eosin staining, consisting of >70% tumor cells in recruited tumor samples were confirmed (Fig. 1). The clinicopathological features of the patients are summarized in Table 2.

3.2. Down-regulation of SOX1 in ESCC

SOX1 mRNA expression was analyzed using relative comparative real-time PCR method. The mean (±SD) of gene expression in ESCC tissues was −0.54 (± 2.89). The lower fold change of gene expression was −4.30, while the upper was 1.67. SOX1 was significantly underexpressed in nearly 25% of tumor samples (9 of 40) with mean (±SD) of −2.48

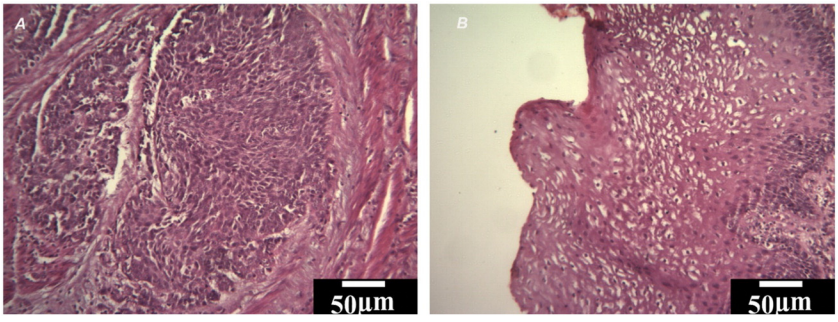

Fig. 1. Histological analysis of tumor (A) and margin normal esophagus tissues (B) using Haematoxylin and Eosin staining. Tumor tissues were analyzed to confirm consisting of >70% tumor cell. Normal tissues of esophagus were also confirmed histologically.

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