Characterization of Tumor-suppressive Function of SOX6 in Human Esophageal Squamous Cell Carcinoma

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Running Title: SOX6 in ESCC

Keywords: ESCC, SOX6, TSG, metastasis, prognosis
Translational interference

Increasing knowledge of genes and their biological pathways associated with cancer development and progression has provided opportunities to develop targeted therapeutics for cancer treatment with higher specificity, more efficiency and safety. Here, we identify SOX6 as a tumor suppressor in esophageal squamous cell carcinoma (ESCC). Loss of SOX6 expression is significantly correlated with poor prognosis in ESCC patients. The tumor-suppressive mechanism of SOX6 is associated with its role in G1/S cell cycle arrest via the up-regulation of p53 and p21WAF1/CIP1, along with the down-regulation of cyclin D1/CDK4, cyclin A and β-catenin. These findings suggest that SOX6 could be used as a prognostic marker and a potential therapeutic target in ESCC.
Abstract

**Purpose:** By using cDNA microarray analysis, we identified a transcriptional factor, SOX6, was frequently down-regulated in ESCC. The aim of this study is to investigate the role of SOX6 in human esophageal cancer development, and to examine the prevalence and clinical significance of SOX6 down-regulation in ESCC.

**Experimental design:** Expressions of SOX6 mRNA in 50 ESCCs and SOX6 protein in 300 ESCCs were investigated by semi-quantitative RT-PCR and immunohistochemistry, respectively. The tumor-suppressive function of SOX6 was characterized by cell growth, foci formation, wound-healing and cell invasive assays, and tumor xenograft experiment. Western blot analysis was applied to detect protein expression level.

**Results:** SOX6 was frequently down-regulated in primary ESCCs in both mRNA level (29/50, 58%) and protein level (149/219, 68.0%), which was significantly associated with the poor differentiation ($P=0.029$), lymph node metastases ($P=0.014$), advanced TNM stage ($P=0.000$) and disease-specific survival ($P<0.001$). Multivariate analysis indicated that the down-regulation of SOX6 ($P=0.000$) was a significant independent prognostic factors for ESCC. Functional studies demonstrated that SOX6 was able to suppress both *in vitro* and *in vivo* tumorigenic ability of ESCC cells. The tumor-suppressive mechanism of SOX6 was associated with its role in G1/S cell cycle arrest by up-regulating expressions of p53 and p21$^{\text{WAF1/CIP1}}$ and down-regulating expressions of cyclin D1/CDK4, cyclin A and $\beta$-catenin.

**Conclusions:** We provided the first evidence that SOX6 is a novel tumor suppressor gene in ESCC development and is a potential prognostic marker in ESCC.
Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most common fatal malignances of the digestive tract (1). Despite advances in diagnosis and multimodality therapies, ESCC continues to carry a grim prognosis (2). The major reason for this poor survival is the fact that ESCC frequently metastasizes to regional and distant lymph nodes, even at initial diagnosis. Recently, the treatment of cancer using molecular targets has brought promising results and attracted more and more attention. However, most of the molecular targets, which were commonly found in other epithelial neoplasias, have failed to demonstrate prognostic value for ESCC (3). Hence, characterization of genes involved in the development and progression of ESCC may lead to the identification of new prognostic markers and therapeutic targets (4, 5).

Systematic analysis of expression levels of thousands of genes by cDNA microarray is an effective approach to identify novel genes and pathways with importance in cancer development and progression, or as targets for new therapies. Recently, genome-wide expression profiles between pooled cDNA from 10 primary ESCCs and their corresponding nontumorous tissues were compared by cDNA microarray containing 47,000 genes. Using an arbitrary cutoff line at Signal Log Ratio $\geq$2.0 or $\leq$-2.0, 185 up-regulated genes and 225 down-regulated genes were detected in ESCC tumors as compared to normal controls (unpublished data). One frequently down-regulated gene in ESCC specimen, SOX6, was further characterized. SOX6, a member of the D subfamily of sex determining region y-related transcription factors, encodes a protein that binds to DNA through a high-mobility-group (HMG) domain and plays critical roles in cell fate determination.
differentiation and proliferation (6-9). Some Sox factors, such as SOX2, SOX4 and SOX9, have been identified as tumor suppressors that were frequently down-regulated in various cancers (10-15). However, to our knowledge, the role of SOX6 in esophageal cancer development and progression has not been explored.

In the present study, the expression pattern of SOX6 in clinical ESCC samples and ESCC cell lines was studied by RT-PCR. Both in vitro and in vivo assays were used to study the tumor suppressive function of SOX6. In addition, the tumor-suppressive mechanism of SOX6 and its clinical significance in ESCC was also investigated.

Materials and Methods

Cell lines and primary tumor specimens. Chinese ESCC cell line HKESC1 was kindly provided by Professor Srivastava (Department of Pathology, The University of Hong Kong, Hong Kong, China), and two Chinese ESCC cell lines (EC18 and EC109) were kindly provided by Professor Tsao (Department of Anatomy, The University of Hong Kong). Six Japanese ESCC cell lines (KYSE30, KYSE140, KYSE180, KYSE410, KYSE510 and KYSE520; ref. 16) were obtained from DSMZ (Braunschweig, Germany), the German Resource Centre for Biological Material. Fifty pairs of primary ESCC tumors and their adjacent nontumorous tissues from the proximal resection margins were collected immediately after surgical resection at Linzhou Cancer Hospital (Henan, China). A total of 300 formalin-fixed and paraffin-embedded ESCCs and their corresponding nontumorous tissue samples were also kindly provided by Linzhou Cancer Hospital. Clinical data of patients included in this study are detailed in Supplementary Table S1 and S2. No patients...
recruited in this study have received any preoperative treatment. Samples used in this study were approved by the individual institutional Committees for Ethical Review of Research involving Human Subjects.

**Semi-quantitative reverse transcription-PCR.** Total RNA was extracted from cell lines and frozen ESCC tissues by the TRIzol reagent (Invitrogen). Reverse transcription of total RNA (2 µg) was done using an Advantage RT for PCR kit (Clontech), and cDNA was subjected to PCR for 28 cycles of amplification with the following pair of primers: SOX6Fw, 5’-GGCGTCCCCCTACCCTGTCATCC and SOX6Rv, 5’-TGCTGCACACCGGCTCCTCAC-TG. GAPDH gene was used as a control.

**Tissue microarray (TMA) and immunohistochemistry (IHC).** TMAs containing 300 pairs of primary ESCC tissue samples and their corresponding nontumorous tissues were constructed as described previously (17). Standard streptavidin-biotin-peroxidase complex method was used for IHC staining (17). Briefly, TMA section was deparaffinized, blocked with 10% normal rabbit serum for 10min, and incubated with anti-SOX6 polyclonal antibody (Novous, 1:75 dilution) overnight at 4°C. The TMA section was then incubated with biotinylated goat anti-rabbit immunoglobulin at a concentration of 1:75 at 37°C for 30min. The status of nuclear expression of SOX6 was assessed by three independent investigators without prior knowledge of clinicopathologic data. Positive expression of SOX6 in normal and malignant ESCC tissues was primarily a nuclear pattern. Because the intensity of staining within each tumor tissue core was mostly homogeneous, the intensity of SOX6 staining was semi-quantitatively evaluated on the basis of criteria used by our previous publication (18): strong positive (scored as 2+), dark brown staining in >50% of normal or
malignant esophageal squamous cells completely obscuring nucleus; weak positive (scored as 1+), any lesser degree of brown staining appreciable in cell nucleus; absent (scored as 0), no appreciable staining in normal or malignant esophageal squamous cells.

**Tumor-suppressive function of SOX6.** To test tumor-suppressive function of SOX6, SOX6 gene was PCR amplified, sequence-verified, cloned into pcDNA3.1(+) vector (Invitrogen), and transfected into ESCC cell line KYSE30 and KYSE510 cells. Stable SOX6-expressing clones (SOX6-30/SOX6-510) were pooled and the SOX6 cDNA was re-sequenced. Blank vector-transfected KYSE30 and KYSE510 cells (Vec-30/Vec-510) were used as control. For foci formation assay, 1×10^3 SOX6-30/SOX6-510 cells or Vec-30/Vec-510 cells were plated in wells of a six-well plate. After 7 days culture, surviving colonies (>50 cells per colony) were counted with Giemsa staining. Triplicate independent experiments were performed.

Cell growth rates of SOX6-30/SOX6-510 and Vec-30/Vec-510 cells were detected by MTT assay. Cells were seeded in 96-well plate at a density of 1×10^3 per well. The cell growth rate was detected using cell proliferation MTT kit (Sigma) according to the manufacturer’s instruction. Triplicate independent experiments were performed.

**Tumor formation in nude mice.** The in vivo tumor suppressive ability of SOX6 was investigated by tumor xenograft experiment. About 2×10^6 SOX6-30 cells or Vec-30 cells were injected s.c. into the right and left hind legs of 4-week-old nude mice (10 mice per group). Tumor formation in nude mice was monitored over a 4-week period. The tumor volume was calculated by the formula $V = 0.5 \times L \times W^2$ (19).

**Migration and invasion assays.** For cell migration assay, SOX6-30/SOX6-510 or...
Vec-30/Vec-510 cells were cultured in a six-well plate until confluent. The cell layer was wounded using a sterile tip. After incubation for 24 hr or 36 hr, the cells were photographed under a phase-contrast microscope. The experiment was performed in triplicate. For invasion assay, SOX6-30 cells or Vec-30 cells were starved with serum free medium for 24 hrs before the assay. Cells (5×10^5) were suspended in 0.5 ml serum-free medium and loaded on the upper compartment of invasion chamber coated with Matrigel (BD Biosciences). The lower compartment was filled with complete medium as chemoattractant. After 24 hr, invasive cells were fixed, stained, and counted under a microscope. Triplicate independent experiments were performed.

**Cell cycle analysis.** SOX6-30/SOX6-510 or Vec-30/Vec-510 cells (1×10^5 to 2×10^5) were cultured in RPMI medium containing 10% fetal bovine serum (FBS). Serum was withdrawn from culture medium when cells were 70% confluent. After 72 hr, 10% FBS was added in the medium for an additional 12 hr. Cells were fixed in 70% ethanol, stained with propidium iodide, and DNA content was analyzed by Cytomics FC (Beckman Coulter).

**Western blot analysis.** Western blotting was done according to the standard protocol with antibodies for SOX6 (Novous), p53, p21^{WAF1/CIP1}, cyclin A, cyclin D1, CDK4 (Cell Signalling Technology) and β-catenin (Santa Cruz Biotechnology).

**Statistical analysis.** Statistical analysis was done with the SPSS standard version 13.0 (SPSS Inc). The correlation between SOX6 expression and clinicopathologic characteristics was analyzed using the chi-square test. Disease-specific survival (DSS) was calculated from the date of diagnosis to the date of cancer-related death or last follow-up. Survival curves was assessed by the Kaplan-Meier method and compared by the log-rank test. Relative risks
of cancer-related death associated with SOX6 expression status and other predictor variables were estimated by univariate analyses. Multivariate survival analysis was carried out on all parameters that were found to be significant on univariate level using the Cox regression model. Differences were considered significant when \( P \) value was less than 0.05.

**Results**

**Down-regulation of SOX6 is frequently detected in ESCCs.** The mRNA expression of SOX6 in nine ESCC cell lines and 50 primary ESCC tumors and their paired nontumorous tissues were studied by semi-quantitative RT-PCR. Expression of SOX6 was observed in all 50 tested nontumorous tissues. However, down-regulation of SOX6 was detected in 29/50 (58%) of primary ESCCs (Fig. 1A). Similarly, down-regulation of SOX6 was detected in 5 of 9 (EC109, HKESC1, KYSE30, 510 and 520) ESCC cell lines (Fig. 1B).

**Down-regulation of SOX6 correlates with poor survival outcome in ESCC.** To investigate the clinical significance of down-regulation of SOX6 in esophageal carcinogenesis, SOX6 expression in protein level was also studied using ESCC tissue microarray containing 300 primary ESCC cases. The clinico-pathologic features of these cases were summarized in Table 1. Positive staining of SOX6 was mainly observed at the nucleus (Fig. 1C) and the expression of SOX6 was classified into absent (scored as 0), weak positive (scored as 1+) and strong positive (scored as 2+) staining. Informative expression of SOX6 was detected in 219 ESCC cases. Non-informative samples included lost samples, unrepresentative samples, samples with too few tumor cells, and samples with inappropriate staining; such were not used in data complication. Normal expression of SOX6 (strong positive staining) was
observed in 205/219 (93.6%) of normal esophageal epithelial cells. Down-regulation of SOX6 (absent and weak positive staining) was detected in 149/219 (68.0%) of informative ESCC cases (Table 1).

The correlation of SOX6 expression with various clinico-pathologic features was investigated and the result showed that down-regulation of SOX6 was significant associated with poor differentiation ($P=0.029$), lymph node metastasis ($P=0.014$) and advanced stage ($P=0.000$, Table 1). Furthermore, log rank test showed that ESCC patients with SOX6 down-regulation (median survival time, 18 months) experienced a shorter disease-specific survival than patients with normal SOX6 expression (median survival time, 37 months; $P<0.001$; Fig. 2). By univariable analysis, down-regulation of SOX6 ($P<0.0001$), poor differentiation ($P=0.012$), presence of lymph node metastasis ($P<0.0001$) and advanced stage ($P<0.0001$) were significant negative prognostic factors for disease-specific survival in ESCC patients (Table 2). Nevertheless, multivariate analysis showed that down-regulation of SOX6 and presence of lymph node metastasis were two independent prognostic predictors for ESCC patients enrolled in this study ($P<0.001$, Table 3).

**SOX6 has strong tumor suppressive ability.** To determine if SOX6 has tumor suppressive ability, SOX6 gene was stably transfected into KYSE30 and KYSE510 cells (SOX6-30/SOX6-510). Empty vector-transfected KYSE30 and KYSE510 cells (Vec-30/Vect-510) were used as control. Expression of SOX6 in SOX6-30 and SOX6-510 was confirmed by RT-PCR or Western blot analysis, respectively (Fig. 3A). Tumor suppressive function of SOX6 was studied by foci formation assay, cell growth assay, and tumor xenograft experiment. Foci formation assay showed that the efficiency of foci...
formation was significantly inhibited ($P<0.001$) in SOX6-transfectants compared with control cells (Fig. 3B). Cell growth assay also revealed that the cell growth rates in SOX6-30 cells were significantly inhibited by SOX6 ($P<0.01$) compared with Vec-30 cells (Fig. 3C). A similar result was shown in SOX6-510 cells (Fig. 3D).

**SOX6 inhibits cell migration and invasion.** As the TMA result showed that down-regulation of SOX6 was closely associated with ESCC metastasis, the effects of SOX6 on cell migration and invasion were studied by wound-healing and cell invasion assays, respectively. Would-healing assay showed that the ectopic expression of SOX6 could dramatically inhibit cell migration ability in SOX6-transfectants compared with control cells (Fig. 4A). Matrigel invasion assay also found that SOX6 could inhibit the invasiveness of ESCC cells, as demonstrated by a significant decrease in the number of invaded cells in SOX6-30 cells compared with Vec-30 control ($P<0.001$, Fig. 4B).

**SOX6 inhibits tumor formation in vivo.** To further explore the *in vivo* tumor suppressive ability of SOX6, tumor formation in nude mouse was performed by injection of SOX6-30 cells (n=10), whereas Vec-30 cells were used as controls. Within 4 weeks, solid tumors were readily visible in left hind legs of all 10 mice (injected with Vec-30 cells), but only observed in 4/10 of mice injected with SOX6-30 cells. In addition, the size of tumors caused by SOX6-30 cells (tumor volume: $385\pm50\text{ mm}^3$) was significantly smaller than tumors ($750\pm37\text{ mm}^3$) induced by Vec-30 cells ($P<0.001$; Fig. 4C). These results suggested that SOX6 had a strong tumor suppressive ability *in vivo.*

**SOX6 arrests cell cycle at G1/S transition.** To elucidate the mechanism underlying growth inhibition by SOX6, flow-cytometry was used to compare cell distributions in cell
cycle between SOX6-transfectants and control cells. The percentage of SOX6-transfectants in G0/G1 phases and in S-phase were significantly increased and decreased, respectively, compared with that in control cells, suggesting that SOX6 was able to inhibit DNA synthesis and G1/S phase transition (Fig. 5A and 5B). To further reveal the potential molecular mechanism of SOX6 in cell cycle arrest, the effects of SOX6 on several key cell cycle regulators, including p53, p21^{WAF1/CIP1}, cyclin A, cyclin D1, CDK4 and β-catenin were investigated by Western blot analysis. Increased expression of p53 and p21^{WAF1/CIP1}, down-regulation of cyclin D1/CDK4 complex, cyclin A and β-catenin were detected in SOX6-30 and SOX6-510 cells, compared with those in control cells (Fig. 5C).

Discussion

The known functions of the SOX protein in development and disease are both diverse and complex (20-22). In this study, we found that SOX6 was frequently down-regulated in ESCC cell lines and primary ESCC tumors. Tissue microarray study showed that the down-regulation of SOX6 was detected in 68% primary ESCCs, which was significantly associated with advanced stage, increased lymph node metastasis and poor survival of patients with ESCC. Multivariate analysis demonstrated that down-regulation of SOX6 could be used as an independent prognostic predictors for ESCC patients. In contrast, it has been reported that high SOX6 expression may be a potential diagnostic marker for some types of brain tumors (23, 24). However, the biological role of high SOX6 expression in brain tumor is still unknown. The contradictory results indicated that SOX6 expression might thus be tissue specific. In this study, the mechanisms underlying silencing of SOX6 in ESCC
cells have also been investigated. However, neither methylation nor mutation of SOX6 were detected (data not shown), suggesting that other mechanisms such as micro-RNA regulation or histone modification might be involved in the inactivation of SOX6.

The tumor suppressive function of SOX6 demonstrated by both in vitro and in vivo assays further supports its role as a TSG in the development and progression of ESCC. The results showed that SOX6 could suppress cell growth, decrease foci formation and cell motility, and inhibit tumor formation in nude mice. Further study revealed that SOX6 was able to inhibit G1/S-phase transition through the up-regulation of p53 and p21<sub>WAF1/CIP1</sub>, and down-regulation of cyclin D1/CDK4 complex and cyclin A in ESCC cancer cells. G1/S phase transition is known to be a major checkpoint for cell cycle progression. The cyclin-dependent kinase inhibitor (CKI), p21<sub>WAF1/CIP1</sub>, serves as a critical negative mediator during this transition through CDK inhibition and regulate the activity of cyclin D-CDK complex, which are essential for S phase entry (25-27). Because an inhibition of CKI activity is one of the factors causing uncontrolled tumor cell growth (27), one possible strategy to control cancer cell proliferation is to induce CKI expression, which would lead to G1 arrest and inhibit tumor growth. Although the tumor suppressor p53 itself is not a CKI, it has been implicated in various cellular processes (28, 29), including induction of G1 arrest by trans-activating its downstream target gene, p21<sub>WAF1/CIP1</sub> (30). Taken together, it is reasonable to speculate that SOX6 is able to induce p53 expression, in turn, activate the expression of p21<sub>WAF1/CIP1</sub>, resulting in its inhibition of cyclin D1/CDK4 activity in human esophageal cancer cells. However, the interaction between the transcription regulator SOX6 and the tumor suppressor p53 need to be further investigated.
In addition to the suppression of cyclin D1/CDK4 complex, our data also showed that ectopic expression of SOX6 resulted in the inhibition of the late G1-phase regulator cyclin A. Expression of cyclin A has been strongly implicated in the control of cell proliferation and prognoses in human cancers (31-33). These findings are complementary to ours and suggest that down-regulation of SOX6 would favor tumor progression.

Recent study by Iguchi et al has demonstrated that SOX6 suppresses cyclin D1 promoter activity by interacting with β-catenin, and its down-regulation induces pancreatic β-cell proliferation (9). Because the Wnt/β-catenin signaling pathway regulates a variety of cellular processes, including cell proliferation, differentiation, development, and apoptosis (34, 35), the suppression of β-catenin signaling by SOX6 may be of biological significance. Previous studies have also shown that some other Sox proteins, including SOX1, SOX3, SOX9 and SOX17, could bind β-catenin and inhibit β-catenin signaling (36-39). In this study, we did found that SOX6 could down-regulate β-catenin expression in ESCC cells, suggesting that the tumor suppressive function of SOX6 might also be associated with the inhibition of β-catenin signal transduction pathway. Interestingly, the oncogenic role of β-catenin in enhancing proliferation could also be inhibited by p53 in response to growth arrest (40-42). Thus, it will be important to know whether SOX6 could exert the tumor-suppressor effect in esophageal cancer through interaction with p53 directly.

In summary, we provided evidences that SOX6 is a tumor suppressor in ESCC, based on the following facts: (1) SOX6 is frequently down-regulated in ESCC tissues; (2) down-regulation of SOX6 was significantly associated with poor prognosis; (3) the introduction of SOX6 into ESCC cells could inhibit cell proliferation and tumor formation in
nude mice; (4) the ectopic expression of SOX6 is able to arrest cell cycle at G1/S checkpoint by up-regulating p53 and p21, down-regulating cyclin D1/CDK4, cyclin A and \( \beta \)-catenin. A better understanding of the tumor suppressive role of SOX6 will significantly improve our knowledge in the development of ESCC, and may lead to a more effective management of ESCC with the inactivation of \( SOX6 \).
Reference


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34. Huelsken J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates.


Figure Legends

Fig. 1. Down-regulation of SOX6 in ESCC. SOX6 expression was frequently down-regulated in primary ESCCs (A) and ESCC cell lines (B) detected by RT-PCR. For primary ESCCs, expression of SOX6 in tumor tissues (T) was compared with their paired nontumorous tissues (N). GAPDH was used as a loading control. C, representative of SOX6 expression in a pair of ESCC (right) and adjacent normal tissue (left) detected by immunostaining with anti-SOX6 antibody (brown). The slide was counterstained with hematoxylin. Original magnification, x200.

Fig. 2. Kaplan-Meier analysis of survival in patients with ESCC. Black, patients with normal SOX6 expression (n=70, median survival 18 months); grey, patients with down-regulation of SOX6 (n=149, median survival 37 months; P<0.001, log-rank test).

Fig. 3. Tumor-suppressive function of SOX6 in ESCC cells. A, expression of SOX6 in SOX6-transfected ESCC cells (SOX6-30/SOX6-510) was confirmed by RT-PCR and Western blot analysis. Empty vector-transfected ESCC cells (Vec-30/Vec-510) were used as controls. B, representative inhibition of foci formation in monolayer culture by SOX6 (left). Quantitative analyses of foci numbers (right). Columns, mean of at least three independent experiments; bars, SD. **, P<0.001 versus controls by using the Student’s t-test. Growth curves of SOX6-30 cells (C) and SOX6-510 cells (D) were compared with control cells by MTT assay. Points, mean of at least three independent experiments; bars, SD. **, P<0.01.
Fig. 4. SOX6 inhibits metastasis of ESCC cells.  

A, the effect of SOX6 on cell migration was determined by wound-healing assay. During a period of incubation, the spreading speed of SOX6-expressing cells along the wound edge was slower than that in control cells.  

B, representative images showed the SOX6-30 cells and Vec-30 cells that invaded through the matrigel. Number of invaded tumor cells was quantified in the right panel. Columns, mean of triplicate experiments. **, *P*<0.001.  

C, tumor growth curves of SOX6-30 cells in nude mice were compared with Vec-30 cells by tumor xenograft experiment. The average tumor volume of SOX6-30 cells versus Vec-30 cells was expressed as mean±SD in 10 inoculated sites for each group. **, *P*<0.001, Student’s *t*-test.

Fig. 5. SOX6 induces G1-S cell cycle arrest. Representative and summary of DNA content detected by flow cytometry showed that the percentage of cells in S phase was much lower in SOX6-30 cells (A) and SOX6-510 cells (B) than that in control cells. Values are the mean±SD of three independent experiments. **, *P*<0.05.  

C, protein expression of p21, p53, β-catenin, cyclin A, cyclin D1, and CDK4 were determined by western blot analysis. GAPDH was used as an internal control.
A

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SOX6

GAPDH

B

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SOX6

GAPDH

C

Non-tumor tissue

ESCC

Qin et al. Figure 1
A

Vec-30
- G1: 42.42%
- S: 40.58%
- G2: 17.00%

SOX6-30
- G1: 50.47%
- S: 36.21%
- G2: 11.32%

Cell cycle distribution (%)

G1 | S | G2
---|---|---
Vec-30 | 40 | 50 | 10
SOX6-30 | 35 | 45 | 20

B

Vec-510
- G1: 35.4%
- S: 42.4%
- G2: 22.2%

SOX6-510
- G1: 45.0%
- S: 35.2%
- G2: 19.8%

Cell cycle distribution (%)

G1 | S | G2
---|---|---
Vec-510 | 35 | 45 | 20
SOX6-510 | 40 | 50 | 10

C

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Table 1. Association between SOX6 expression and clinicopathologic characteristics of patients with ESCC ($n = 219$)

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<td>30(30.9)</td>
<td>0.086</td>
</tr>
<tr>
<td>Female</td>
<td>122</td>
<td>82(66.4)</td>
<td>40(33.6)</td>
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</tr>
<tr>
<td>Tumor cell differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>25</td>
<td>13(52.0)</td>
<td>12(48.0)</td>
<td>7.416</td>
</tr>
<tr>
<td>Moderate</td>
<td>141</td>
<td>93(66.0)</td>
<td>48(34.0)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>53</td>
<td>43(79.2)</td>
<td>10(20.8)</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes metastasis(N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>114</td>
<td>69(60.5)</td>
<td>45(39.5)</td>
<td>6.167</td>
</tr>
<tr>
<td>N1</td>
<td>105</td>
<td>80(76.2)</td>
<td>25(23.8)</td>
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</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>3(60.0)</td>
<td>2(40.0)</td>
<td>17.277</td>
</tr>
<tr>
<td>II</td>
<td>131</td>
<td>76(58.0)</td>
<td>55(42.0)</td>
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</tr>
<tr>
<td>III</td>
<td>83</td>
<td>70(84.3)</td>
<td>13(15.7)</td>
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</tr>
<tr>
<td>General classification</td>
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<tr>
<td>medullary type</td>
<td>119</td>
<td>82(68.9)</td>
<td>37(31.1)</td>
<td>1.950</td>
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<tr>
<td>ulcerative type</td>
<td>61</td>
<td>43(70.5)</td>
<td>18(29.5)</td>
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</tr>
<tr>
<td>sclerotic type</td>
<td>14</td>
<td>10(71.4)</td>
<td>4(28.6)</td>
<td></td>
</tr>
<tr>
<td>mushroom type</td>
<td>25</td>
<td>14(56.0)</td>
<td>11(44.0)</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.05$
Table 2. Univariate Cox regression analysis of factors possibly influencing disease-specific survival in patients with ESCC

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX6</td>
<td>0.338</td>
<td>0.242-0.471</td>
<td>0.000*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.206</td>
<td>0.912-1.595</td>
<td>0.188</td>
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<tr>
<td>Gender</td>
<td>1.052</td>
<td>0.793-1.395</td>
<td>0.726</td>
</tr>
<tr>
<td>Differentiation</td>
<td>1.349</td>
<td>1.067-1.706</td>
<td>0.012*</td>
</tr>
<tr>
<td>pN factor</td>
<td>1.859</td>
<td>1.401-2.467</td>
<td>0.000*</td>
</tr>
<tr>
<td>TNM stage</td>
<td>2.148</td>
<td>1.622-2.843</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*P < 0.05
Table 3. Multivariate Cox regression analysis for factors possibly influencing disease-specific survival in patients with ESCC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratio</th>
<th>95% Confidence interval</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX6</td>
<td>0.360</td>
<td>0.257-0.506</td>
<td>0.000*</td>
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<tr>
<td>Differentiation</td>
<td>1.220</td>
<td>0.963-1.548</td>
<td>0.100</td>
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<tr>
<td>pN factor</td>
<td>1.733</td>
<td>1.301-2.309</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* $P < 0.05$
Clinical Cancer Research

Characterization of Tumor-suppressive Function of SOX6 in Human Esophageal Squamous Cell Carcinoma

Xin-Yuan Guan, Yanru Qin, Hong Tang, et al.

Clin Cancer Res Published OnlineFirst November 17, 2010.

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