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Original Article

SOX1 suppresses cell growth and induces apoptosis by regulating Wnt/β-catenin signaling pathway in gastric cancer

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Abstract: Gastric cancer (GC) is the fourth most common malignancy diagnosis and the second leading cause of cancer related mortalities worldwide. The role of SOX1 has been explored in several human cancers. However, its effects in gastric cancer have never been reported. In our study, we investigated the expression level of SOX1 and its biological function in GC. Firstly, we found that SOX1 is down-regulated GC tissues and cell lines on both mRNA and protein levels. Next, we demonstrated that ectopic expression of SOX1 significantly inhibited proliferation and induced apoptosis in GC cell lines. Furthermore, we showed that SOX1 may exert its tumor suppressive role by regulating Wnt/β-catenin signaling pathway in gastric cancer. In conclusion, our findings suggest that SOX1 may play important roles in the development of gastric cancer and SOX1 may serve as a potential therapeutic target in the treatment of gastric cancer.

Keywords: SOX1, proliferation, apoptosis, Wnt/β-catenin

Introduction

Gastric cancer (GC) is the fourth most common malignancy diagnosis and the second leading cause of cancer related mortalities worldwide, especially in Asian countries including china [1]. It is estimated that 464,000 men and 273,000 women died from gastric cancer in 2011 [2]. Although the mortality rate of gastric cancer has decreased in numerous areas of the world due to the progress of early detection and new treatment strategies during the past few decades, most patients have found a distant metastasis when first diagnosed with gastric cancer because of the asymptomatic presentation in the early stage and the clinical outcome of advanced GC patients is extremely poor [3, 4]. Although peri- and post-operative chemo or chemoradiation is helpful for clinical outcome, surgery still remains the only curative treatment for gastric cancer patients [5]. Therefore, to explore some novel molecule involved in the initiation and development of gastric cancer may be helpful for early detection and treatment of GC patients.

The family of sex-determining region Y (SRY)-box (SOX) proteins contain a highly conserved high-mobility group (HMG) DNA binding domain and play crucial roles in embryonic and postnatal development [6, 7]. A large number of studies demonstrated that different members of SOX gene family may play different roles in the development of human cancers. For example, SOX3 and SOX10 are identified as oncogenes in esophageal squamous cell carcinoma and ovarian epithelial tumors, respectively, and high expression of these two proteins were significantly associated with poor overall survival [8, 9]. SOX9 is reported to be over-expressed in lung adenocarcinoma and ectopic overexpression of SOX9 in the lung adenocarcinoma cell line resulted in a marked increase in cell proliferation, migration and invasion [10]. SOX1 is belongs to Group B of the SOX gene family [11] and its tumor suppressive role through regulating Wnt/β-catenin signaling pathway has been explored in many human cancers such as hepatocellular carcinoma [12], cervical cancer [13] and nasopharyngeal carcinoma [14]. However,

the role of SOX1 in gastric cancer and the underlying mechanism still remains unknown.

In this study, we investigated the expression level of SOX1 in gastric cancer tissues and cell lines firstly. Then, we conducted some in vitro assays to explore the role of SOX1 in gastric cancer cells proliferation and apoptosis. Finally, we find that SOX1 may suppress gastric cancer development by regulating Wnt/β-catenin signaling pathway. Our findings indicate that SOX1 is involved in the development of gastric cancer and may be applied as a new therapeutic target in gastric cancer patients.

Materials and methods

Clinical specimens

24 primary gastric cancer tissue specimens and the matched non-tumor gastric tissues (5 cm away from the tumor border) were collected from GC patients who had underwent surgery at the Department of General Surgery, Huaihe Hospital of HeNan University, during the period from 2013 to 2015. All tissues were frozen in liquid nitrogen immediately following the surgical resection and kept at -80°C until use. Both cancer tissues and non-tumor tissues were confirmed by two pathologists independently, No patients received adjuvant treatment including radiotherapy or chemotherapy prior to surgery. This study was approved by the Ethics Committees of our hospital and written informed consent was obtained from each patients.

Cell culture

Human gastric cancer cell lines MKN-45, MGC-803, HGC-27 andSGC-7901 were all obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The human gastric epithelial mucosa cell line GES-1 was purchased from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI-1640 medium (HyClone) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA), 100 U/ml of penicillin and 100 μg/ml of streptomycin. Cells were maintained at 37°C in a humidified incubator containing 5% CO_2.

Plasmid construction and cell transfection

The SOX1 cDNA was cloned into the pcDNA3.1 to construct the SOX1 expression plasmid and the empty vector was used as the control (mock). Cell transfection was performed using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol, and the transfection efficiency were confirmed by qRT-PCR and western blot.

Quantitative real-time-PCR

Total RNA was extracted from tissue samples or cells using TRIzol reagent (Invitrogen) following the manufacturer's instruction. For Quantitative Real-time PCR (qRT-PCR), 2 μg of total RNA from each sample was used to synthesize cDNA with SuperScript® III RT (Invitrogen) and an oligo-dT primer. qRT-PCR was performed with SYBR Green master mix (Invitrogen) using a 7500 Fast Real-Time Sequence detection system (ABI). The relative expression level of SOX1 was calculated by using the 2-ΔΔCt method and GADPH was used as internal control. The primers for SOX1 were forward 5'-AAGGTGAAGGTCGGAGTCAAC-3', reverse 5'-GGGGTCATTGATGGCAACAATA-3'. The primers for GAPDH were forward 5'-CATCTTCTTTTGCGTCGCCA-3', reverse 5'-TTAAAAGCAGCCCTGGTGACC-3'. All experiments were conducted in triplicate.

Western blot analysis

Total protein was extracted from tissue samples or whole cells with RIPA lysis buffer (Beyotime, Shanghai, China) on ice and the protein concentration was quantified using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Inc.). Equal amounts of protein samples were subjected to SDS-PAGE and then transferred to PVDF membranes (Billerica, MA, USA). The PVDF membranes were then blocked with 5% non-fat dry milk at room temperature for 1 h and incubated with primary antibody at 4°C overnight. After washing three times with PBS supplemented with 0.05% Tween 20 (Biosharp), the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 2 h and protein bands were visualized using ECL system (Pierce, Rockford, IL, USA). The primary antibodies were all purchased from cell signaling technology (Danvers,

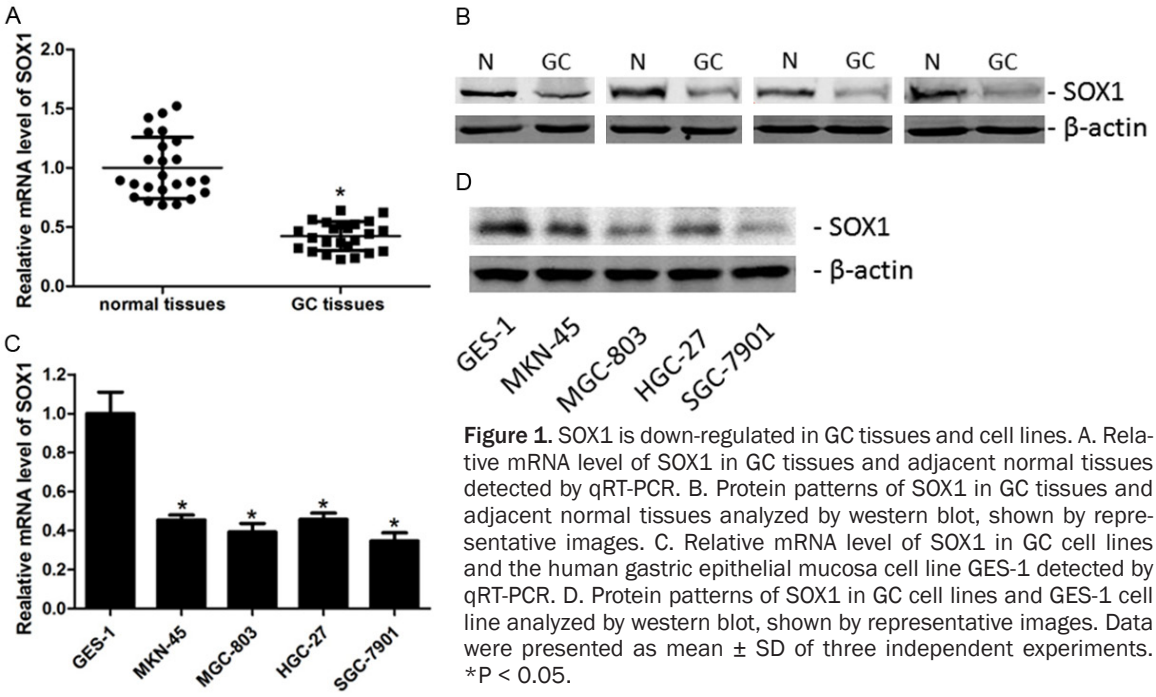

Figure 1. SOX1 is down-regulated in GC tissues and cell lines. A. Relative mRNA level of SOX1 in GC tissues and adjacent normal tissues detected by qRT-PCR. B. Protein patterns of SOX1 in GC tissues and adjacent normal tissues analyzed by western blot, shown by representative images. C. Relative mRNA level of SOX1 in GC cell lines and the human gastric epithelial mucosa cell line GES-1 detected by qRT-PCR. D. Protein patterns of SOX1 in GC cell lines and GES-1 cell line analyzed by western blot, shown by representative images. Data were presented as mean ± SD of three independent experiments. *P < 0.05.

MA, USA). The secondary antibodies and the primary antibody against β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Three independent experiments were performed.

MTT assay

Cell proliferation was measured by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay according to the manufacturer's protocol. Briefly, after effectively transfection, cells were harvested and seeded into 96-well plates at a density of 2 × 103 cells per well. After incubation at 37°C for different time (0 h, 24 h, 48 h, 72 h, 96 h), the cells were further incubated with 20 μL MTT (5 mg/ml, Sigma) for 4h at 37°C and 150 μL of DMSO was added to stop the reaction. Finally, the optical density was determined at a wavelength of 490 nm. This experiment was repeated at least three times.

Cell apoptosis assay

Cell apoptosis was assessed using flow cytometry with FITC-Annexin V Apoptosis Detection Kit (BD Biosciences, CA, USA) following the manufacturer's protocol. Briefly, treated cells were harvested and washed twice with cold PBS. Then, cells were treated with Annexin V and propidium iodide (PI) for 15 min in the dark. Apoptosis cells were examined by FACS Caliber flow cytometer (BD Bioscience). Tests were performed in triplicate.

Statistical analysis

Statistical analysis was performed with SPSS version 17.0 (SPSS Inc., Chicago, IL). All data were presented as mean ± SD. The significance of differences between groups was compared by Student's t test (two groups) or one-way analysis of variance (ANOVA, more than two groups). $P < 0.05$ was considered to be significant.

Results

SOX1 is down-regulated in GC tissues and cell lines

To investigate the potential functional role of SOX1 in gastric cancer, we first detected the expression pattern of SOX1 in GC tissues and cell lines on both mRNA and protein levels. In GC tissue samples, we found that the SOX1 mRNA levels and protein levels are both obviously lower than those in the adjacent non-tumor gastric tissues (**Figure 1A**, **1B**). Consistently, our data revealed that SOX1 is down regulated in GC cell lines compared with the

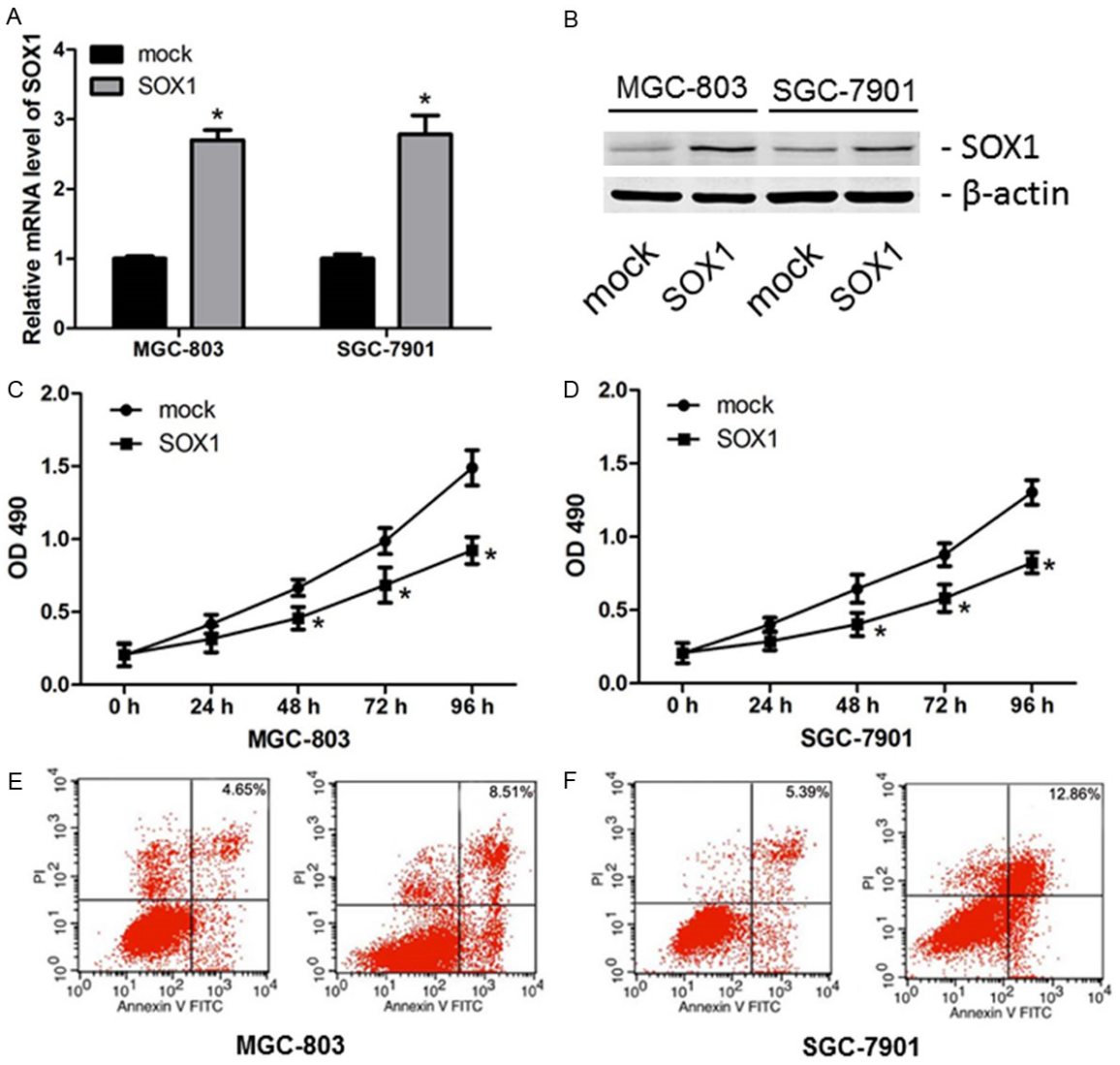

Figure 2. Ectopic expression of SOX1 inhibited proliferation and induced apoptosis in GC cells. A, B. Relative SOX1 levels in transfected GC cell line MGC-803 and SGC-7901, determined by qRT-PCR and western blot, respectively. C, D. Cell proliferation of MGC-803 and SGC-7901 determined by MTT after SOX1 overexpression. E, F. Cell apoptosis of MGC-803 and SGC-7901 analyzed by flow cytometry after SOX1 overexpression. Data were presented as mean ± SD of three independent experiments. *P < 0.05.

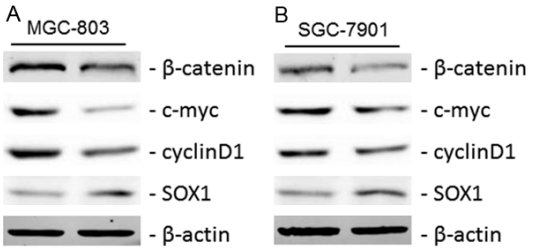

Figure 3. SOX1 may function as a tumor suppressor by regulating Wnt/β-catenin signaling pathway in gastric cancer. A. Expression of β-catenin, c-myc and cyclin D1 in MGC-803 after SOX1 overexpression. B. Expression of β-catenin, c-myc and cyclin D1 in SGC-7901 after SOX1 overexpression.

human gastric epithelial mucosa cell line GES-1 (**Figure 1C**, **1D**). These indicate that SOX1 is down-regulated in GC tissues and cell lines, and it may play important roles in the development of gastric cancer.

Ectopic expression of SOX1 inhibited proliferation and induced apoptosis in GC cells

Since the down-regulation of SOX1 is observed in both GC cells and tissues, we transfected GC cells (MGC-803 and SGC-7901) with SOX1 overexpression plasmid or empty vector (mock) to explore the effect of SOX1 on GC cells prolif-

eration and apoptosis. The transfect efficiency was confirmed on both mRNA and protein levels (**Figure 2A**, **2B**). Then, results of MTT assay showed that ectopic expression of SOX1 significantly inhibited GC cells proliferation (**Figure 2C**, **2D**). Besides, data from Cell apoptosis assay revealed that overexpression of SOX1 induced apoptosis in GC cells (**Figure 2E**, **2F**).

SOX1 may function as a tumor suppressor by regulating Wnt/β-catenin signaling pathway in gastric cancer

After demonstrated SOX1 as a tumor suppressor in gastric cancer, we further explored the underlying mechanism. Since the Wnt/β-catenin signaling pathway is crucial for cell proliferation, we supposed that SOX1 may regulate Wnt/β-catenin signaling pathway in gastric cancer. Results of western blot showed that overexpression of SOX1 significantly downreulated β-catenin, c-myc and cyclin D1 in GC cells (**Figure 3A**, **3B**).

Discussion

The balance between cell growth and death is critical for the maintenance of normal tissue architecture [15]. Gastric cancer cells have the high proliferation ability and low apoptosis rate, resulting in its malignant behaviors. As one of the most important regulators of tumor cells proliferation, Wnt/β-catenin signaling pathway is crucial in the development of gastric cancer. Three signaling pathways (nuclear factor-κB, Wnt/β-catenin, and proliferation/stem cell) are deregulated in more than 70% of the patients diagnosed with gastric cancer, while activation of the Wnt/β-catenin signaling is found in about 30% to 50% of gastric cancer tissues and in many kinds of gastric cancer cell lines [16].

Apart from SOX1, several members of SOX gene family have been demonstrated to regulate Wnt/β-catenin signaling pathway in human cancer development. For example, Chan et al. showed that Sox7 is a negative regulator of Wnt/β-catenin signaling pathway through impeding the transcriptional machinery of β-catenin/TCF/LEF-1 transcriptional complex in endometrial cancer [17]. Li et al. reported that SOX2 improves metastasis of breast and prostate cancer cells by promoting epithelial-to-mesenchymal transition (EMT) through WNT/β-catenin [18]. Thu et al. found that SOX15 may exert its tumor suppressive effects by regulating Wnt/β-catenin signaling pathway in pancreatic cancer [19]. In our study, we proved that SOX1 is involved in the pathogenesis of gastric cancer. SOX1 is down-regulated in GC tissues and cell lines. Besides, Ectopic expression of SOX1 inhibited proliferation and induced apoptosis in GC cells. Furthermore, we showed that SOX1 may exert its tumor suppressive role by regulating Wnt/β-catenin signaling pathwayin gastric cancer.

SOX1 may play its roles in human disease through multiple independent pathways such as attenuating Notch signaling by directly binds to the Hes1 promoter and suppresses Hes1 transcription, regulating Wnt/β-catenin signaling pathway by binding to beta-catenin, and up-regulatting transcription of the proneural bHLH transcription factor neurogenin 1 (ngn1) [20]. So, we will further investigate other mechanisms involved in the tumor suppressive role of SOX1 in gastric cancer. Besides, further studies are required to reaveal whether SOX1 affect gastric cancer cells migration and invasion.

In summary, the current study showed for the first time that SOX1 is down-regulated gastric cancer tissues and cell lines. In addition, SOX1 can inhibit proliferation and induce apoptosis by regulating Wnt/β-catenin signaling pathway in gastric cancer. Our findings suggest that SOX1 may play important roles in the development of gastric cancer and SOX1 may serve as a potential therapeutic target in the treatment of gastric cancer.

Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Guggenheim DE and Shah MA. Gastric cancer epidemiology and risk factors. J Surg Oncol 2013; 107: 230-236.

[3] Crew KD and Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol 2006; 12: 354-362.
[4] Jiang Y and Ajani JA. Multidisciplinary management of gastric cancer. Curr Opin Gastroenterol 2010; 26: 640-646.
[5] Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, Andreozzi F, Ventriglia J, Savastano B, Mabilia A, Lieto E, Ciardiello F and De Vita F. Treatment of gastric cancer. World J Gastroenterol 2014; 20: 1635-1649.
[6] Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, Vivian N, Goodfellow P and Lovell-Badge R. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature 1990; 346: 245-250.
[7] Chew LJ and Gallo V. The Yin and Yang of Sox proteins: Activation and repression in development and disease. J Neurosci Res 2009; 87: 3277-3287.
[8] Li K, Wang RW, Jiang YG, Zou YB and Guo W. Overexpression of Sox3 is associated with diminished prognosis in esophageal squamous cell carcinoma. Ann Surg Oncol 2013; 20 Suppl 3: S459-466.
[9] Kwon AY, Heo I, Lee HJ, Kim G, Kang H, Heo JH, Kim TH and An HJ. Sox10 expression in ovarian epithelial tumors is associated with poor overall survival. Virchows Arch 2016; 468: 597-605.
[10] Wang X, Ju Y, Zhou MI, Liu X and Zhou C. Upregulation of SOX9 promotes cell proliferation, migration and invasion in lung adenocarcinoma. Oncol Lett 2015; 10: 990-994.
[11] She ZY and Yang WX. SOX family transcription factors involved in diverse cellular events during development. Eur J Cell Biol 2015; 94: 547-563.
[12] Tsao CM, Yan MD, Shih YL, Yu PN, Kuo CC, Lin WC, Li HJ and Lin YW. SOX1 functions as a tumor suppressor by antagonizing the WNT/beta-catenin signaling pathway in hepatocellular carcinoma. Hepatology 2012; 56: 2277-2287.
[13] Lin YW, Tsao CM, Yu PN, Shih YL, Lin CH and Yan MD. SOX1 suppresses cell growth and invasion in cervical cancer. Gynecol Oncol 2013; 131: 174-181.
[14] Guan Z, Zhang J, Wang J, Wang H, Zheng F, Peng J, Xu Y, Yan M, Liu B, Cui B, Huang Y and Liu Q. SOX1 down-regulates beta-catenin and reverses malignant phenotype in nasopharyngeal carcinoma. Mol Cancer 2014; 13: 257.
[15] O'Brien LE and Bilder D. Beyond the niche: tissue-level coordination of stem cell dynamics. Annu Rev Cell Dev Biol 2013; 29: 107-136.
[16] Chiurillo MA. Role of the Wnt/beta-catenin pathway in gastric cancer: An in-depth literature review. World J Exp Med 2015; 5: 84-102.
[17] Chan DW, Mak CS, Leung TH, Chan KK and Ngan HY. Down-regulation of Sox7 is associated with aberrant activation of Wnt/b-catenin signaling in endometrial cancer. Oncotarget 2012; 3: 1546-1556.
[18] Li X, Xu Y, Chen Y, Chen S, Jia X, Sun T, Liu Y, Li X, Xiang R and Li N. SOX2 promotes tumor metastasis by stimulating epithelial-to-mesenchymal transition via regulation of WNT/beta-catenin signal network. Cancer Lett 2013; 336: 379-389.
[19] Thu KL, Radulovich N, Becker-Santos DD, Pikor LA, Pusic A, Lockwood WW, Lam WL and Tsao MS. SOX15 is a candidate tumor suppressor in pancreatic cancer with a potential role in Wnt/beta-catenin signaling. Oncogene 2014; 33: 279-288.
[20] Kan L, Israsena N, Zhang Z, Hu M, Zhao LR, Jalali A, Sahni V and Kessler JA. Sox1 acts through multiple independent pathways to promote neurogenesis. Dev Biol 2004; 269: 580-594.