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Sox2 silencing effects on proliferation and apoptosis of bladder cancer cells

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Received April 2, 2018, accepted May 8, 2018

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Pharmazie 73: 469-473 (2018) *doi: 10.1691/ph.2018.8450*

Background: Bladder cancer is the sixth most common malignant tumor in men in the world. Our study aimed to investigate the silencing effect of Sox2 on human bladder cancer cell proliferation and apoptosis. *Methods*: T24 cells were selected and divided in control, scrambled siRNA and Sox2-siRNA groups. Morphological changes in T24 cells were observed after transfection. qRT-PCR and Western blot were performed to observe mRNA and protein expression of apoptosis-related proteins. MTT and flow cytometry were used to detect cell proliferation, cell cycle and apoptosis. *Results*: Cells grew adhered to the wall and mainly showed a spindle shape with a clear nucleolus in control and scrambled siRNA groups. Increases in cell number in early apoptosis and metaphase cells in apoptosis were observed in Sox2-siRNA group compared with control and scrambled siRNA groups. Sox2-siRNA group showed Sox2 down-regulation relative to that in control and scrambled siRNA groups. There were no significant differences in cell proliferation, cell cycle distribution or apoptosis between control and scrambled siRNA groups. Cell proliferation ability decreased significantly in Sox2-siRNA group, while cell number in G0/G1 stage, cell number of apoptotic cells and caspase-3 and caspase-9 expressions increased significantly. *Conclusion*: The results showed that Sox2 silencing could inhibit human bladder cancer cell proliferation and promote cell apoptosis.

1. Introduction

Bladder cancer is the sixth most common malignant tumor in men in the world, with an estimated 429 800 morbidity and about 165100 deaths each year (Zhu et al. 2017). Chemotherapy and surgical treatment of bladder cancer often fail due to the presence of cancer stem cells. Tumor stem cells play a key role in tumor recurrence and metastasis, and their presence makes it very difficult to treat tumors (Cui et al. 2017). However, on the other hand, the characteristics that bladder cancer stem cell can drive cancer development suggest that they can be used as an ideal target of anti-cancer drugs, in order to control the occurrence and development of tumors from the very beginning. More molecular biology research on cancer stem cells are always needed to advance and support current clinical approaches to bladder cancer.

Sox2 is an important stem cell marker gene and belongs to the SRY-like HMG Box gene family. It contains DNA binding region HMG, which is located on chromosome 3q26 and regulates stem cell gene Oct-3/4, Nanog, Fgf-4, Utf1 and Lefty1 expression. Sox2 is located in the center of stem cell signaling regulatory network and plays an important role in maintaining stem cell self-renewal and multidirectional differentiation (Mu et al. 2017). Epidemiological studies have found that upregulation of Sox2 is associated with poor prognosis of lung and esophageal cancers and may serve as an independent predictor of prognosis (Maehara et al. 2018). Further study also found that overexpression of Sox2 can promote lung cancer cell growth and migration, and Sox2 can also promote cervical cancer cell proliferation (Kim et al. 2015).

Currently, the upregulation of Sox2 in bladder cancer and bladder cancer stem cells has been confirmed (Migita et al. 2017), but the effects of Sox2 expression difference on biological functions of bladder cancer cells were not yet fully studied. Therefore, to confirm the relationship between Sox2 and the proliferation and apoptosis of T24 cells, this study used RNA interference to silence genes in order to observe how Sox2 silencing affects cell proliferation and apoptosis of T24 cells.

2. Investigations and results

2.1. Sox2 silencing effect on T24 cell morphology

Forty eight hours after transfection, cells were observed by inverted phase contrast microscopy, the cells in the control group and the scrambled siRNA group grew in a spindle-shaped manner with clear nucleoli and showed no significant difference from normal cells. In contrast, the number of cells in Sox2-siRNA group was significantly reduced, with wrinkled cells, more cytoplasmic particles, cells with concentrated nuclei, blistering cell membrane, cell debris and even apoptotic bodies, and many cells shifting from static adherent growth to a suspended state (Fig. 1).

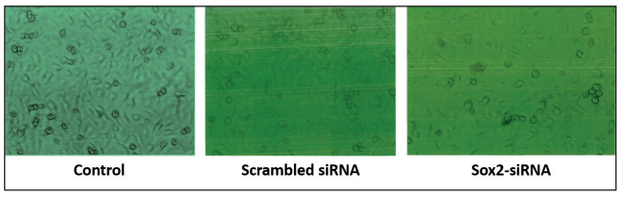

Fig. 1: Effects of SOX2 gene silencing on the morphology of T24 cells after transfection (× 200).

2.2. Sox2 silencing effect on Sox2 expression in T24 cells after transfection

RNA was isolated from T24 cells 48 h after transfection. qRT-PCR results showed that there was no significant difference in Sox2 mRNA expression between the Scramble siRNA group and the control group ($P > 0.05$), while in the Sox2-siRNA group it was significantly lower than in the other two groups (all $P < 0.05$) (Fig. 2A). Western blotting was used to detect the protein expression of Sox2 in each group, indicating that Sox2 protein expression was consistent with the expression of mRNA in each group (Fig. 2B, C).

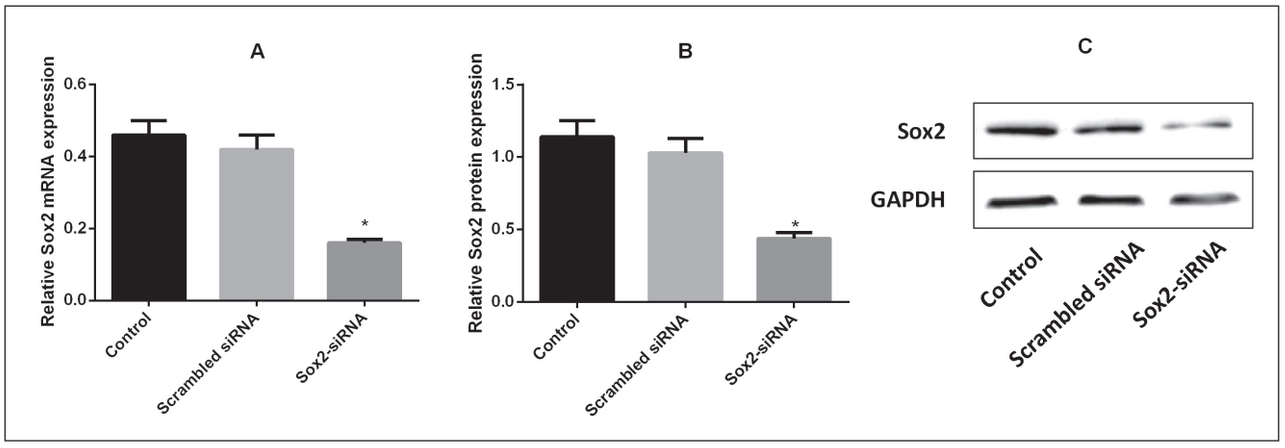

Fig. 2: Effects of SOX2 gene silencing on SOX2 expression in T24 cells.
A: SOX2 mRNA expression detected with qRT-PCR; B: Statistical analysis of SOX2 protein detected with Western blotting; C: SOX2 protein expression detected with Western blotting

2.3. Sox2 silencing effect on T24 cell proliferation

The results of MTT assay at 24 h, 48 h, and 72 h after transfection (Table 3) showed that there was no significant difference in cell survival rate between the scrambled siRNA group and the control group regardless of time (all $P > 0.05$), cell viability of the Sox2-siRNA group at the same time point was significantly lower than that of other groups ($P < 0.05$). The cell proliferation rate of the Sox2-siRNA group gradually decreased at 24, 48 and 72 h, which were 81.33±9.77%, 70.15±7.63% and 54.82±5.17%, respectively (all $P < 0.05$). This suggests that Sox2 silencing could inhibit cell proliferation in T24 cell lines.

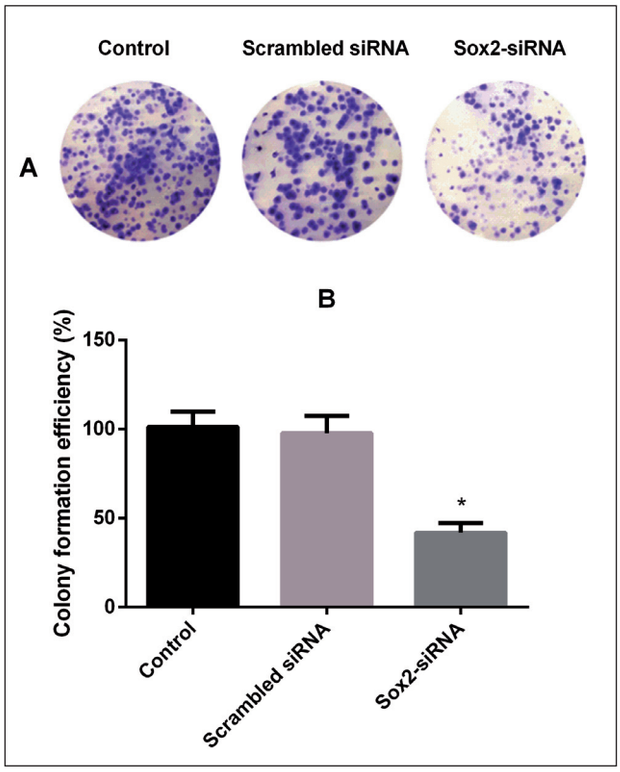

Fig. 3: Effects of SOX2 gene silencing on T24 cell clones in each group after transfection.
Note: A: figure of clone formation after transfection; B: T24 cell clone formation efficiency

2.4. Sox2 silencing effect on clone formation

The results of colony formation assays after transfection showed that there was no significant difference between scrambled siRNA group and control group ($P > 0.05$) (Fig. 3A), while the Sox2-siRNA group had much lower colony-forming ability than the other two groups (all $P < 0.05$) (Fig. 3B). This demonstrates that Sox2 silencing could inhibit colony formation of T24 cells.

2.5. Sox2 silencing effect on T24 cell cycle and apoptosis

24, 48, and 72 hours after T24 cells were transfected, the changes of cell cycle in each group were detected by flow cytometry. Results showed that compared with the control group, there was no difference in cell cycle distribution of scrambled siRNA group. However, the Sox2-siRNA group showed a significant change, and most of cells were arrested in the G0/G1 phase, that is, the number of cells in the G1 phase was significantly increased, while the number of cells in the S phase and the G2/M phase was significantly decreased ($P < 0.05$). This indicates that Sox2 silencing prevented G1 phase cells from transitioning to S phase (Figs. 4A, B, C). According to flow cytometry, there was no significant difference in apoptotic rate between control group and scrambled siRNA group (all $P > 0.05$). The apoptotic rate of Sox2-siRNA group was significantly higher than that of the other two groups (all $P < 0.05$), and the apoptosis rate increased at a later time point, indicating that the Sox2 silencing continued to promote T24 cell apoptosis (Fig. 4D).

2.6. Sox2 silencing effect on apoptosis signaling pathway-related protein expressions

Forty eight hours after transfection, qRT-PCR was performed to detect signaling pathway-related gene expressions. Results showed that there was no significant difference in the mRNA expression of caspase-3 and caspase-9 between the scrambled siRNA group and the control group, however, it was higher in the Sox2-siRNA group than in the other two groups. (all $P < 0.05$) (Fig. 5A). The protein expression of cleaved caspase-3 and cleaved caspase-9 detected by Western blotting was consistent with that detected by qRT-PCR (Fig. 5B, C).

3. Discussion

As one of the most common malignant tumors of the urinary system, bladder cancer ranks ninth in the world in terms of the incidence of malignant tumors and ranks second in the world in male genitourinary tumors (Azevedo et al. 2017). Although 75% of cases are classified as NMIBC (non-muscle invasive bladder cancer) with a high 5-year survival rate, another 25% of MIBC (muscle invasive bladder cancer) are prone to metastasis after the

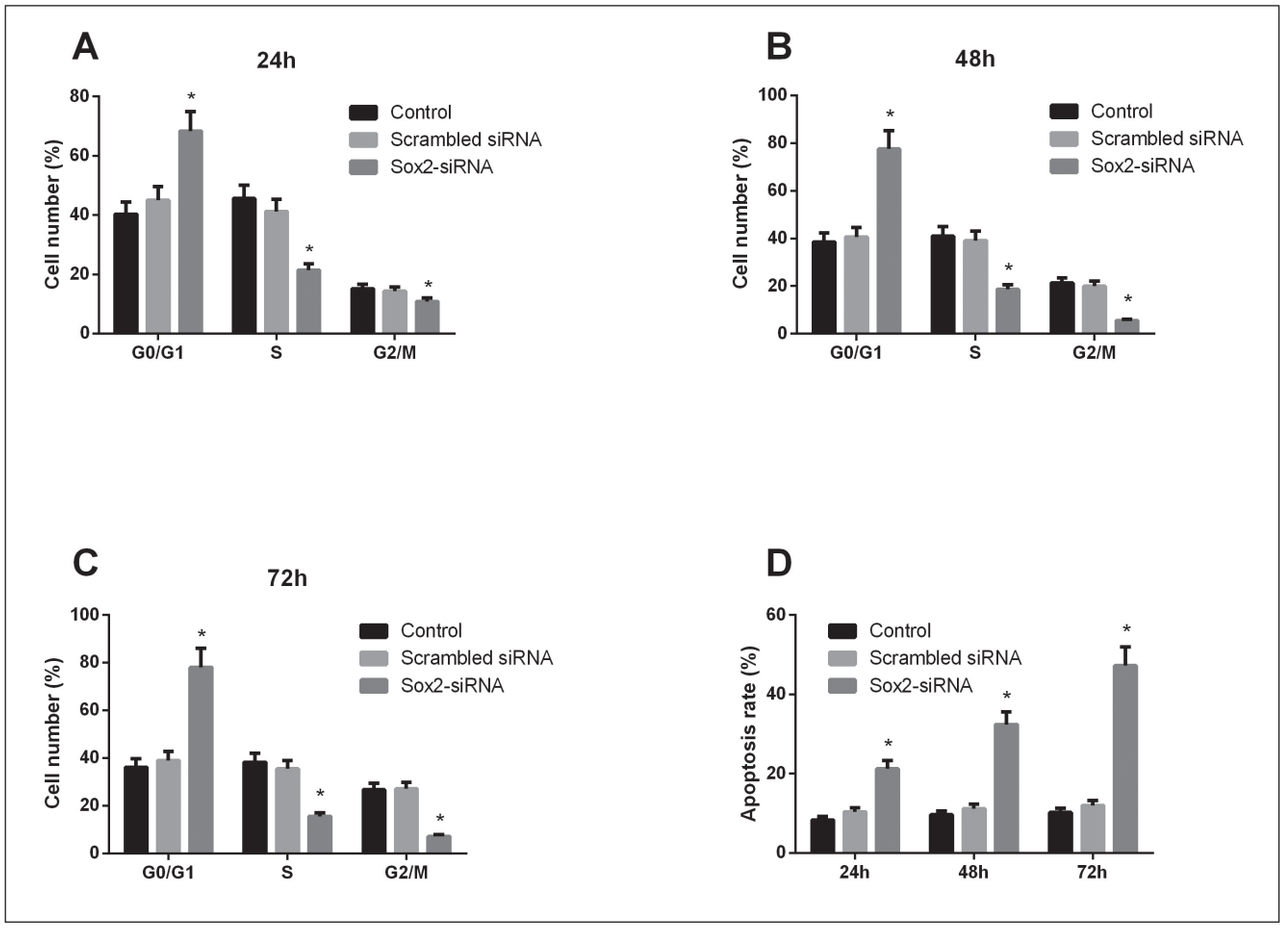

Fig. 4: Effects of SOX2 gene silencing on cell cycle and apoptosis of T24 cells after transfection.
Note: A, cell number 24 h after transfection; B, cell number 48 h after transfection; C, cell number 72 h after transfection; D, cell apoptosis rate of each group 24, 48 and 72 h after transfection.

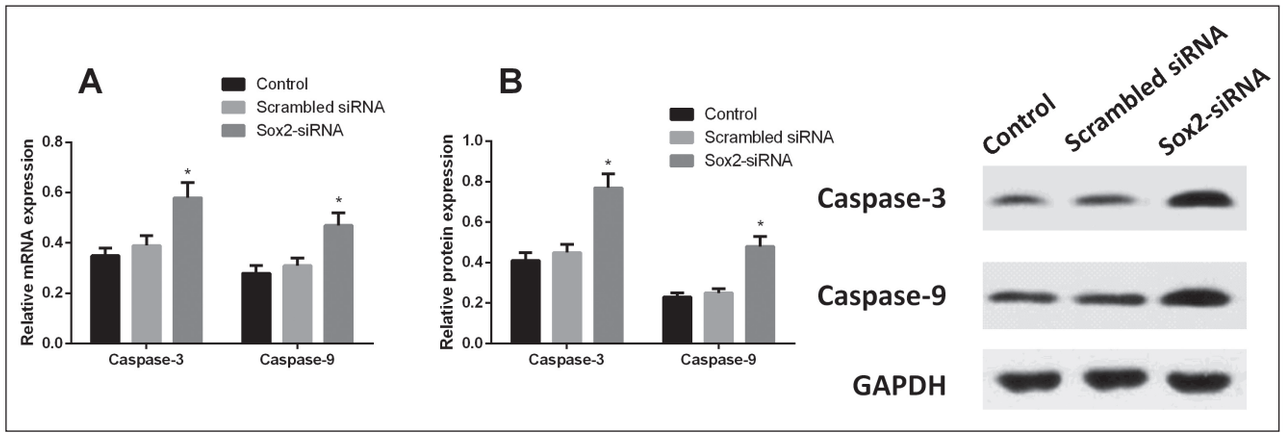

Fig. 5: Effects of SOX2 gene silencing on the expression of signaling pathway-related proteins, including caspase-3 and caspase-9, in T24 cells. A: Caspase-3 and caspase-9 mRNA expression was detected with qRT-PCR; B: statistical analysis result showing cleaved caspase-3 and cleaved caspase-9 protein; C: expression of cleaved caspase-3 and cleaved caspase-9 protein detected with Western blotting; *, $P < 0.05$ compared with the control group;

first active treatment (Passos et al. 2017). Therefore, searching for markers of early diagnosis and prognosis of bladder cancer has become a hot area in recent years.

Previous studies have revealed that Sox2 plays a critically important role in tumor progression in bladder cancer cells, thus making the inhibition of SOX2 a new therapy option for anti-tumor invasion and metastasis (Fang and Kitamura 2018). It would be of great significance to patients' overall survival and quality of life if further efforts could be made investigate the effects of SOX2 on the invasion and metastasis of T24 cells in bladder cancer.

RNA interference is described as a response to double-stranded RNA that leads to sequence-specific posttranscriptional gene silencing (Guo et al. 2017). In regard to siRNA, it is incorporated into a nuclease complex called the RNA-induced silencing complex (RISC) that targets and cleaves mRNA complementary to the siRNA and thus lowers its expression (Ma et al. 2018). Having

selected the T24 bladder cancer cell line and used RNA interference to silence genes, this study found that the SOX2-siRNA group exhibited remarkably lower expression of SOX2 mRNA and protein than the control group and the scrambled siRNA group. Therefore, it is assumed that, when induced by SOX2-siRNA, the RISC-mediated cleavage of SOX2-RNA results in the degradation of mRNA and leads to a decrease in mRNA expression, which further causes a decrease in SOX2 expression due to the lack of its corresponding mRNA.

The cell cycle refers to a process that spans from the end of the last cell division to the end of the current cell division, including the 4 phases G1, S, G2, and M, in which cyclin is of vital importance for regulation. In addition, cell proliferation relies on the recurrence of the cell cycle (Yadav et al. 2017). SOX2 gene silencing can cause a decrease in the expression of cyclins such as cyclins A and B, cdc2 and p34, thus inhibiting cell proliferation by impeding the cell division of the T24 cell line, which is stuck in G0/G1 phase (Sheng et al. 2018). It was also revealed in the study that silencing SOX2 can prevent cells in G1 phase from transitioning into S phase by inhibiting the proliferation and clone formation of the T24 cell line in bladder cancer. Zhu et al. (2017) found that SOX2 was over-expressed in bladder cancer cell lines relative to nonmalignant bladder cell lines and normal bladder tissues and that knockdown of SOX2 expression inhibited bladder tumor cell proliferation through the induction of apoptosis or cell cycle arrest, which is consistent with our findings (Zhu et al. 2017).

Cell apoptosis is a complicated process that is regulated by various factors and signaling pathways, with the two main apoptotic pathways being the intrinsic, or mitochondrial, pathway and the extrinsic pathway (Liao et al. 2017). The activation of caspases is a key factor in the signaling pathway that induces cell apoptosis, and the most important caspases are caspase-3, which is often referred to as the pre-caspase for the caspase cascade, and caspase-9 which is regarded as the execution caspase in cell apoptosis (Perdigao et al. 2018). Our results also show that SOX2 silencing could promote T24 cell apoptosis, and caspase-3/9 expressions were both upregulated, which is consistent with previous findings.

This study came to the conclusion that SOX2 could inhibit bladder cancer cell proliferation and promote cell apoptosis, which may offer new treatment options in bladder cancer. However, the study was only conducted *in vitro*, and can therefore not simulate a complicated physiological environment, and cannot be extrapolated to studies *in vivo*; additionally, the study did not investigate the regulatory mechanism of SOX2. Therefore, more studies need to be carried out to explore the function of SOX2 and its mechanism in the complicated operating system. Furthermore, deeper investigation into the role of SOX2 in a wider range of tumors is worthwhile.

4. Experimental

4.1. Cell culture and grouping

T24 cell lines, bought from Procell Life Science & Technology Co.,Ltd (Wuhan, China), were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% inactivated fetal bovine serum (FBS, Gibco Company, Grand Island, NY, USA), 100 units/ml penicillin (General Electric Company, USA) and 100 mg/ml streptomycin (General Electric Company, USA) at 37 °C and stored in an incubator containing 5% CO_2 and constant temperature (Thermo Fisher Scientific, California, USA). Samples were digested with 0.25% pancreatic enzymes when cells reached 80% confluency, (Gibco Company, Grand Island, NY, USA). Cells were divided into three groups, including Sox2-siRNA group, scrambled-siRNA group and control group. Scrambled-siRNA had same nucleotide composition as Sox2-siRNA while without distinct homology. Sox2-siRNA and scrambled-siRNA sequences are shown in Table 1, and they were synthesized by Shanghai GenePharma Company (Shanghai, China) after alignment, using basic local alignment search tool (BLAST) from the National Center of Biotechnology Information (NCBI).

Table 1: siRNA sequences

Gene	Sequence
Sox2-siRNA	5'-CCUGUGGUUACCUCUUCCUCCCACU-3'
Scrambled siRNA	5'-AAAAACGGTAGATGCATCAGC-3'

4.2. Cell transfection

According to the transfection reagent instructions, T24 cells were plated 24 h before transfection and were continuously incubated until 60% ~ 70% confluency. Then, cells were transfected. Cells were serum starved in FBS-free DMEM solution for 1 h before transfection. In accordance with Lipofectamine 2000 kit instruction (Invitrogen Inc., Carlsbad, CA, USA), transfection compounds were prepared for each well. Control group was incubated with serum-free, antibiotic-free medium, while Sox2-siRNA group was incubated with serum-free, antibiotic-free medium containing Sox2 siRNA (final concentration was 20 μmol/L), which was enclosed in lipidosomes (Invitrogen Inc., Carlsbad, CA, USA); scrambled-siRNA group was incubated with serum-free, antibiotic-free medium containing scrambled-siRNA (final concentration was 20 μmol/L) that was enclosed in lipidosomes. Transfected cell cultures were blocked for 4 h in serum-free medium, after which 10% FBS was added, and cells were cultured in a 5% CO_2 incubator at 37 °C.

4.3. Cell observation using phase contrast microscopy

Forty eight hours after transfection, the T24 cells were observed under a CKX41 Olympus inverted microscope (Olympus Optical Co., Ltd., Tokyo, Japan). The condenser phase plate was adjusted to fit with the magnification of the objective. The assistant lens cone was moved, and the condenser phase plate was adjusted to make two identical aureoles for viewing the image with the eyepiece lens replaced by an assistant lens. The assistant lens was changed to an eyepiece lens, and a phase contrast image was finally formed after that. The cells were removed from culture solution and placed on objective table of an inverted phase contrast microscope. Cell growth was observed, and photos were taken with round fields focused on cells.

4.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

One Step SYBR ® PrimeScript ™ (Takara, Japan) qPCR Kit and 7300 real-time fluorescence quantitative PCR instrument were used to detect GFAP, NR1 and NR2B mRNA expression according to the kit instruction. Reaction conditions were as follows: 95 °C for 30 s; 95 °C for 5 s; 60 °C for 30 s, 40 cycles. The relative mRNA expression levels of GFAP, NR1 and NR2B were calculated by $2^{-\Delta\Delta Ct}$ method. β-actin was used as internal reference and primer sequences were shown in Tabel 2.

4.5. Western blotting

A bicinchoninic acid (BCA) kit (Sigma-Aldrich, Customer Support PO Box 14508 St. Louis, MO 63178 USA) was used to detect the protein expression. After sample buffer was added to the proteins (each well, 30 μg per sample), proteins were boiled at 95 °C for 10 min. Then, the proteins were separated using 10% polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to polyvinylidene fluoride (PVDF) membranes with 100 V transfer-molded voltage lasting for 45 to 70 min. Then, samples were incubated at room temperature for 1 h with 5% bovine serum albumin (BSA), and were incubated with primary antibodies (1: 1000 dilution), at 4 °C overnight. After that, samples were washed with tris-buffered saline Tween 20 (TBST) 3 times (5 min/time). The corresponding secondary antibody was added for incubation at room temperature for 1 h. After which membranes were washed 3 times (5 min/time). Development was completed with chemiluminescence reagents. GAPDH was used as an internal reference. Bands were visualized with a Bio-Rad Gel Doc EZ imager (Life Science Research, Education, Process Separations, Food Science, 2000 Alfred Nobel Drive, Hercules, California 94547, USA). ImageJ software (National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892) was applied to analyze the intensity of the target bands.

4.6. MTT assay

Cells in the logarithmic growth phase were collected and inoculated into a 96-well plate after the cell concentration was adjusted to 5×10^6 cells/mL. The PG group was treated with 40 mg/L prodigiosin and the 5-FU group was treated with 0.35 mg/L 5-FU. After the cells adhered to the bottom of the plate, 10 μL MTT was added to each well after 24, 48, 72 and 96 h of culture. Subsequently, the cells were cultured for another 4 h in the incubator. After the culture solution was discarded, 150 μL dimethyl sulfoxide (DMSO) was added, and the plates were allowed to shake for 10 min in the dark. The absorbance value at a wavelength of 490 nm was detected.

4.7. Clone formation assay

Agar plates were prepared with RPMI 1640 medium supplemented with 10% FBS and 0.6% agar that were added into the 6-well plates. The plates were kept at room temperature for 10 min. After the plates were solidified, a 0.5-mL T24 cell suspension (concentration: 2×10^3 cells/mL) in RPMI 1640 medium supplemented with 10% FBS was added. The colony formation of the cells was observed after 14 d in culture at 37 °C. The number of colonies was counted by randomly selecting 5 horizons in each group. Colony forming efficiency (CFE %) was defined as the ratio of the number of colonies formed in culture to the number of cells inoculated.

4.8. Flow cytometry

In order to establish the proportion of cells in each phase of the cell cycle, cells were trypsinized for 3 min, centrifuged and washed once with PBS. The cell pellet was suspended and fixed on ice for 15 min with 1 ml of cold 70% ethanol. The cells were subsequently centrifuged and the cell pellet suspended in 1 ml of propidium iodide (PI) solution (0.05 mg/mL PI, 0.02 mg/mL RNase, 0.3% NP40 1mg/mL sodium citrate) for 1 h at 4 °C. Flow cytometry analysis was performed using a FAC Sort flow cytometer

Table 2: Primer sequences

Gene	Forward	Reverse
Sox2	5'-CATCACCCACAGCAAATGAC-3'	5'-CAAAGCTCCTACCGTACCACT-3'
Caspase-3	5'-TGGCCAATTCTGCCATAAGC-3'	5'-TGGTTCATCCCCATTGACTGT-3'
Caspase-9	5'-GGGCTCACTCTGAAGACCTG-3'	5'-CCCGAGTGAGACTTCTGGAC-3'
GAPDH	5'-TCCTGTGGCATCCACGAAACT-3'	5'-GAAGCATTTGCGGTGGACGAT-3'

Table 3: Cell viability of each group (%)

Group	Control group	Scrambled siRNA group	Sox2-siRNA group
Cell viability at 24 h	98.33±8.84%	97.56±8.72%	76.24±7.14%*
Cell viability at 48 h	97.96±7.95%	96.45±8.74%	68.17±6.23%*#
Cell viability at 72 h	98.15±7.27%	95.33±9.50%	51.97±5.52%*#&

Notes: * indicative of a difference from the control group, $P < 0.05$; # indicative of a difference from the 24 h time point, $P < 0.05$; & indicative of a difference from the 48 h time point, $P < 0.05$.

4.9. Statistical analysis

All data were analyzed by GraphPad Prism version 6 statistical software. Measurement data were expressed by mean±standard deviation. The t test was used for comparisons between two groups. One-way analysis of variance was applied for comparisons between multiple groups. Statistical significance was assumed for $P < 0.05$.

Disclosure statement: The authors declare that they have no conflicts of interest with the contents of this article.

References

Azevedo R, Peixoto A, Gaiteiro C, Fernandes E, Neves M, Lima L, Santos LL, Ferreira JA (2017) Over forty years of bladder cancer glycobiology: Where do glycans stand facing precision oncology? Oncotarget 8: 91734-91764.

Cui Y, Cui CA, Yang ZT, Ni WD, Jin Y, Xuan YH (2017) Gli1 expression in cancer stem-like cells predicts poor prognosis in patients with lung squamous cell carcinoma. Exp Mol Pathol 102: 347-353.

Fang D, Kitamura H (2018) Cancer stem cells and epithelial-mesenchymal transition in urothelial carcinoma: Possible pathways and potential therapeutic approaches. Int J Urol 25: 7-17.

Guo JE, Hu Z, Li F, Zhang L, Yu X, Tang B, Chen G (2017) Silencing of histone deacetylase SlHDT3 delays fruit ripening and suppresses carotenoid accumulation in tomato. Plant Sci 265: 29-38.

Kim BW, Cho H, Choi CH, Ylaya K, Chung JY, Kim JH, Hewitt SM (2015) Clinical significance of OCT4 and SOX2 protein expression in cervical cancer. BMC Cancer 15: 1015.

Liao P, Yang D, Liu D, Zheng Y (2017) GLP-1 and ghrelin attenuate high glucose/high lipid-induced apoptosis and senescence of human microvascular endothelial cells.Cell Physiol Biochem 44: 1842-1855.

Ma W, Chen C, Liu Y, Zeng M, Meyers BC, Li J, Xia R (2018) Coupling of microRNA-directed phased small interfering RNA generation from long noncoding genes with alternative splicing and alternative polyadenylation in small RNA-mediated gene silencing. New Phytol 217: 1535-1550.

Maehara R, Fujikura K, Takeuchi K, Akita M, Abe-Suzuki S, Karbanova J, Corbeil D, Itoh T, Kakeji Y, Zen Y (2018) SOX2-silenced squamous cell carcinoma: a highly malignant form of esophageal cancer with SOX2 promoter hypermethylation. Mod Pathol 38: 83-92.

Migita T, Ueda A, Ohishi T, Hatano M, Seimiya H, Horiguchi SI, Koga F, Shibasaki F (2017) Epithelial-mesenchymal transition promotes SOX2 and NANOG expression in bladder cancer. Lab Invest: doi: 10.1038/labinvest.2017.17.

Mu P, Zhang Z, Benelli M, Karthaus WR, Hoover E, Chen CC, Wongvipat J, Ku SY, Gao D, Cao Z, Shah N, Adams EJ, Abida W, Watson PA, Prandi D, Huang CH, de Stanchina E, Lowe SW, Ellis L, Beltran H, Rubin MA, Goodrich DW, Demichelis F, Sawyers CL (2017) SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. Science 355: 84-88.

Passos GR, Camargo JA, Ferrari KL, Saad MJA, de Mattos AC, Reis LO (2017) Intravesical thalidomide boosts bacillus Calmette-Guerin (BCG) in non-muscle invasive bladder cancer treatment. Med Oncol 35: 3.

Perdigao G, Lopes MS, Marques LB, Prazeres P, de Sousa Gomes K, de Oliveira RB, Xavier Pinto MC, de Souza-Fagundes EM (2018) Novel nitroaromatic compound activates autophagy and apoptosis pathways in HL60 cells. Chem Biol Interact 283: 107-115.

Sheng L, Wan B, Feng P, Sun J, Rigo F, Bennett CF, Akerman M, Krainer AR, Hua Y (2018) Downregulation of survivin contributes to cell-cycle arrest during postnatal cardiac development in a severe spinal muscular atrophy mouse model." Hum Mol Genet 27: 486-498.

Yadav S, Kujur PK, Pandey SK, Goel Y, Maurya BN, Verma A, Kumar A, Singh RP, Singh SM (2017) Antitumor action of 3-bromopyruvate implicates reorganized tumor growth regulatory components of tumor milieu, cell cycle arrest and induction of mitochondria-dependent tumor cell death. Toxicol Appl Pharmacol 339: 52-64.

Zhu F, Qian W, Zhang H, Liang Y, Wu M, Zhang Y, Zhang X, Gao Q, Li Y (2017) SOX2 is a marker for stem-like tumor cells in bladder cancer. Stem Cell Reports 9: 429-437.