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Original Article

The role of SOX18 in nasopharyngeal carcinoma: implications for prognosis and therapy

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Abstract: Objective: To investigate the cellular function of SOX18 in nasopharyngeal carcinoma (NPC) by analyzing its effects on tumor cell proliferation, apoptosis, migration and invasion, and to verify its expression and prognostic significance by clinical samples, thereby providing a basis for precise diagnosis and treatment. Methods: SOX18 expression was analyzed in NPC cell lines and clinical samples. Gene silencing techniques were utilized to reduce SOX18 expression in NPC cells, followed by assays to evaluate cell proliferation, apoptosis, migration, and invasion. Additionally, changes in the Wnt/β-catenin signaling pathway were examined. Results: High SOX18 expression was correlated with poor survival in NPC patients. Silencing SOX18 significantly inhibited cell proliferation, increased apoptosis, and suppressed migration and invasion capabilities. Furthermore, SOX18 silencing downregulated key genes and proteins associated with the Wnt/β-catenin signaling pathway. Conclusion: SOX18 plays a critical role in NPC progression by affecting key cellular behaviors. Targeting SOX18 may offer new therapeutic strategies and improve prognostic assessments for NPC patients, highlighting its potential as a valuable molecular marker for cancer treatment.

Keywords: NPC, SOX18, proliferation, apoptosis, migration, invasion

Introduction

The SOX gene family encompasses a group of transcription factor-encoding genes with highly conserved sequences [1, 2], playing pivotal roles in biological processes such as cell differentiation [3], proliferation [4], and embryonic development [5]. These genes are characterized by their SRY-related HMG-box [6], a DNA-binding domain that enables them to regulate the expression of other genes crucial for cellular function and development [7]. In cancer research, aberrant expression of SOX family members is implicated in the onset, progression, and prognosis of various tumors [1, 8]. Among them, SOX18, a member of the SOX family, has garnered increasing attention for its potential role in multiple malignancies [9-11].

SOX18 expression is closely linked to tumor cell capabilities [12]. In laryngeal cancer studies, downregulation of SOX18 has been shown to significantly inhibit cell processes [13]. Conversely, overexpression of SOX18 promotes these cellular behaviors, enhancing the malignancy of the tumor [14]. This phenomenon suggests that SOX18 may influence tumor cell growth and survival by modulating genes or signaling pathways associated with the cell cycle and apoptosis [15-18]. The underlying mechanism SOX18's effect involves the regulation of target genes that control critical aspects of cell division [19, 20] and programmed cell death [9]. By influencing these pathways, SOX18 contributes to tumorigenesis and tumor progression.

Additionally, SOX18 is implicated in tumor angiogenesis [21], the process of new blood vessel formation that supplies tumors with necessary nutrients and oxygen [22]. In cervical cancer cell lines, SOX18 is a key gene in the Hedgehog signaling pathway [23], which, while crucial in embryonic development, also plays a significant role in the biological processes of various cancers [24, 25]. SOX18 expression,

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regulated by this pathway, promotes cell migration and invasion in vitro, further underscoring its role in tumor angiogenesis [26]. Tumor angiogenesis is crucial for tumor growth and metastasis, as it ensures the provision of nutrients and oxygen to tumors [27, 28]. By promoting angiogenesis, SOX18 facilitates tumor metastasis to other parts of the body.

The level of SOX18 expression is associated with the staging, grading, and prognosis of various tumors [29, 30]. For instance, in ovarian cancer [31], elevated SOX18 expression correlates with the presence of residual disease, advanced stages, and poor patient prognosis. This indicates that SOX18 expression could assist clinicians in predicting disease progression and prognosis, aiding in the development of more tailored treatment plans. By predicting patient outcomes based on SOX18 levels, clinical assessments can be more precise, enabling personalized treatment strategies.

In hepatocellular carcinoma [16, 32, 33], SOX18 expression is associated with poor patient prognosis, and its downregulation can inhibit tumor cell proliferation, migration, and invasion. Inhibiting SOX18 expression or function could effectively suppress tumor growth and metastasis, offering potential treatment options for cancer patients. Targeting SOX18 could involve the development of small molecules [34-36], antibodies [37], or gene therapy approaches [38, 39] designed to specifically inhibit its activity. Such targeted therapies would aim to disrupt the pathways and processes regulated by SOX18, thereby halting tumor progression and potentially leading to tumor regression.

Although the role of SOX18 is gradually being elucidated in various tumors, its function in NPC remains largely unclear. NPC is a malignant tumor originating in the nasopharynx, characterized by a complex pathogenesis involving multiple genes and signaling pathways [40, 41]. Increasing research on SOX family genes in NPC suggests that they may play either oncogenic or tumor-suppressive roles in NPC development [42-44]. For example, high expression of SOX5 in NPC is associated with poor prognosis [44, 45], indicating a complex regulatory network within the SOX family, where different members may have distinct, and potentially opposing, roles in cancer biology.

Investigating SOX18's role in NPC is crucial for advancing scientific and clinical knowledge. Understanding SOX18's expression and functions could reveal its role in NPC cell growth, metastasis, and molecular mechanisms, potentially identifying new diagnostic and therapeutic targets. As a molecular marker, SOX18 could help predict NPC prognosis and treatment response, enhancing personalized treatment strategies. Additionally, targeting SOX18 might offer more effective, selective therapies with fewer side effects [11]. Research in other tumors suggests inhibiting SOX18 could suppress NPC growth and metastasis, highlighting its significant potential for improving NPC patient outcomes [46].

The roles of SOX18 in various tumors, including NPC, offer new avenues for research. In this study, we employed various experimental techniques, such as gene knockdown and signaling pathway analyses, to explore SOX18's specific roles and molecular mechanisms in NPC. Additionally, analyzing clinical samples could validate SOX18 expression and its prognostic relevance in NPC patients, providing a solid foundation for clinical applications.

Material and methods

Cell lines and culture

In this research, we employed a variety of cell lines to investigate the biological mechanisms of NPC. These cell lines comprised NP-69, a normal nasopharyngeal epithelial cell line, alongside three different NPC-derived cell lines, C666-1, HK-1, and 5-8f, all obtained from Pricella (Wuhan Pricella Biotechnology Co., Ltd.). To support cell growth and metabolism, all cell lines were cultured in RPMI-1640 medium (Gibco, Cat# 11875-093), supplemented with 10% fetal bovine serum (FBS, Gibco, Cat# 16000-044) to provide essential growth factors, and 1% penicillin-streptomycin (Gibco, Cat# 15140-122) to prevent bacterial contamination. The cultures were maintained under optimal laboratory conditions, specifically at 37°C with a 5% CO_2 atmosphere and high humidity to ensure physiological pH levels and support cell viability. Subculturing was performed every 2-3 days, depending on cell confluency, to maintain cells in the logarithmic growth phase, which is crucial for reproducible experimental data.

Gene expression analysis

TCGA (https://portal.gdc.cancer.gov/) was used to examine SOX18 expression patterns in different tumors. Data from the GTEx database was used to supplement TCGA data when fewer than five samples from paracancerous tissues were available. The "fdr" algorithm was used to adjust the *p*-value.

RNA extraction and quantitative reverse transcription PCR (RT-PCR)

RNA was extracted using TRIzol reagent (Invitrogen, Cat# 15596-026), following a protocol optimized for high yield and purity. Cells were directly lysed on the culture dish by applying 1 mL of TRIzol reagent per 10 cm^2 of culture area, ensuring complete cell disruption. The lysate was homogenized by repeatedly passing it through a pipette. RNA extraction was performed by adding chloroform to the lysate, followed by vortexing, centrifugation to separate the phases, RNA precipitation with isopropanol, washing with ethanol, air-drying, and dissolution in RNase-free water.

To synthesize complementary DNA (cDNA), PrimeScript RT Reagent Kits (Takara, Cat# RR037A) were utilized according to the manufacturer's guidelines. Quantitative RT-PCR was carried out using the SYBR Green Master Mix (Applied Biosystems, Cat# 4367659). PCR reaction mixtures were prepared with SYBR Green Master Mix, specific primers for SOX18, C-myc, β-catenin, and TCF4, cDNA, and RNase-free water, with GAPDH as an internal control, and cycling conditions optimized for denaturation, amplification, and melting curve analysis to ensure specificity.

siRNA transfection

To explore the function of SOX18 in NPC cells, gene knockdown was executed using siRNA technology. The si-SOX18 sequence (5'-GGAUGUGGAGAGAGUUUAU-3') was specifically designed for optimal silencing efficiency. Lipofectamine 2000 was used to produce siRNA-Lipofectamine complex. These complexes were then gently pipetted into each well containing the NPC cells, and the plates were swirled gently to ensure uniform distribution across the cell surface. After incubating for 6 hours, the culture medium was refreshed to maintain optimal cell growth. Cells were harvested 48 hours post-transfection for downstream analyses, ensuring sufficient knockdown efficiency for the study.

Western blot

To isolate proteins, cells were rinsed twice with chilled PBS solution and lysed in RIPA buffer (Thermo Scientific, Cat# 89901), supplemented with protease and phosphatase inhibitors (Roche, Cat# 04693132001 and 04906837001, respectively). Post-lysis, the samples were incubated on an ice bed for 30 minutes prior to centrifugation. The resulting supernatant, containing the total protein extract, was collected, and protein concentration was determined using the BCA Protein Assay Kit (Pierce, Cat# 23225).

For Western blot analysis, equal amounts of protein were separated by SDS-PAGE electrophoresis. Membranes were blocked in TBST for 1 hour at room temperature to prevent non-specific binding. The membranes were then incubated overnight at 4°C with primary antibodies: SOX18 (Abcam, Cat# ab227680, diluted to 1:1000), C-myc (Cell Signaling Technology, Cat# 5605, diluted to 1:1000), β-catenin (Cell Signaling Technology, Cat# 8480, diluted to 1:1000), TCF4 (Cell Signaling Technology, Cat# 2569, diluted to 1:1000), and p-GSK3β (Ser9) (Cell Signaling Technology, Cat# 9323, diluted to 1:1000). GAPDH (Cell Signaling Technology, Cat# 5174, 1:2000) was used as the internal loading control. After three washes with TBST, the membranes were incubated with HRP-conjugated anti-rabbit IgG (Cell Signaling Technology, Cat# 7074, diluted to 1:5000) or HRP-conjugated anti-mouse IgG (Cell Signaling Technology, Cat# 7076, diluted to 1:5000) for 1 hour at room temperature. After three additional washes with TBST, the protein bands were detected using an enhanced chemiluminescence (ECL) system (Thermo Scientific, Cat# 34580) and visualized using a ChemiDoc MP imaging system (Bio-Rad).

Cell viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8, Dojindo, Cat# CK04), strictly adhering to the guidelines provided by the producer. After seeding, the cells were allowed to adhere to the well surfaces over-

night. At designated time points (24, 48, 72, and 96 hours), the plates were incubated with CCK-8 reagent at 37°C in a humidified 5% CO_2 environment for 2 hours. The optical density (OD) at 450 nm was measured using a spectrophotometer (Bio-Rad, Model 680).

Colony formation assay

The colony formation assay was performed as follows: post-transfection, cells in the logarithmic growth phase were digested with trypsin, resuspended in complete medium, and counted to prepare a cell suspension. Subsequently, 800 cells per well were seeded into 6-well culture plates for each experimental group, with three replicates per group, using complete medium containing 10% FBS. The plates were gently shaken to evenly distribute the cells. Cells were incubated under standard conditions, with medium changes every three days. Colony size and cell conditions were monitored under a microscope. Once the majority of colonies reached over 50 cells, the supernatant was discarded, and the cells were washed once with PBS. The colonies were then fixed in 1 mL of 4% paraformaldehyde at 4°C for 60 minutes, washed with PBS, and stained with 0.1% crystal violet for 15 minutes. After staining, the cells were washed with PBS, and the colonies were photographed and counted for analysis.

Apoptosis assay

To evaluate programmed cell death, known as apoptosis, the Annexin V-FITC Apoptosis Detection Ki (BD Biosciences, Cat# 556547) was employed. Forty-eight hours post-transfection, cells were stained and incubated according to the manufacturer's protocol. Apoptotic cell percentages were determined by flow cytometry, and the results were analyzed using FlowJo software.

Wound healing assay

For the wound healing assay, cells were initially seeded into 6-well culture plates and allowed to proliferate until they reached a confluence level of 90-100%. A uniform scratch was made in the cell monolayer using a sterile 200 μL pipette tip. Subsequently, the cells were rinsed twice with PBS to remove any debris and incubated in serum-deprived RPMI-1640 medium. Images of the wound area were obtained at 0, 24, and 48-hour intervals using an inverted microscope model (Olympus; CKX53). Wound closure was calculated using the following formula: Wound Closure (%) = (initial wound width - wound width at time point)/initial wound width × 100%.

Transwell invasion assay

Transwell chambers with an 8 μm pore size (Corning, Cat# 3422) were used for the invasion assays. After preparing and hydrating the matrix adhesive spreading plate, cells were seeded into the upper chamber and incubated for 24 hours. Following incubation, the cells were fixed with paraformaldehyde, stained with crystal violet, and the number of invaded cells was counted.

Statistical analysis

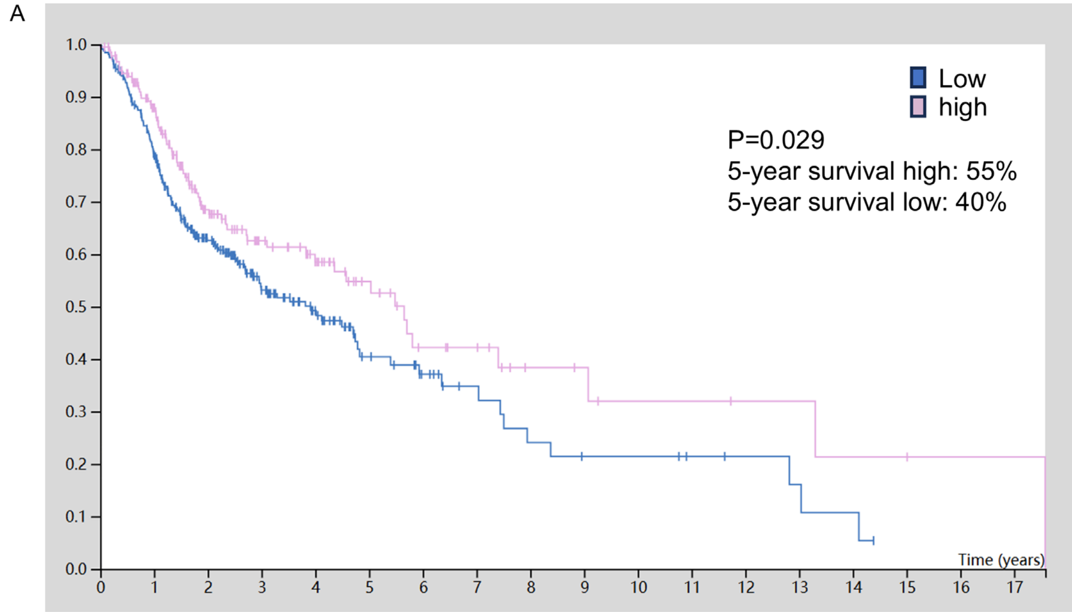
Statistical analyses were conducted using GraphPad Prism software, version 8.0. The data are presented as mean values ± standard deviation (SD). For comparisons between two groups, Student's t-test was employed. Results with p-values < 0.05 were considered statistically significant.

Results

SOX18's impact on head and neck cancer survival

Using gene expression data from the Human Protein Atlas (HPA), we investigated the role SOX18 gene expression in head and neck cancer, especially NPC. The results demonstrated a significant impact of SOX18 expression on the survival rates of patients with head and neck cancer, as shown in **Figure 1A**. Notably, patients with high SOX18 expression had a 5-year survival rate of 55%, whereas those with lower levels had a survival rate of just 40%.

Expanding our investigation to include a broader range of cancer types, we observed notable disparities in SOX18 gene expression between tumor tissues and the surrounding healthy tissues. Particularly striking were the differences noted in head and neck cancer cases, as illustrated in **Figure 1B**. These findings propose that the distinctive expression patterns of SOX18 gene across various cancer types could be of substantial clinical relevance.


A
Low
high
P=0.029
5-year survival high: 55%
5-year survival low: 40%
Time (years)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
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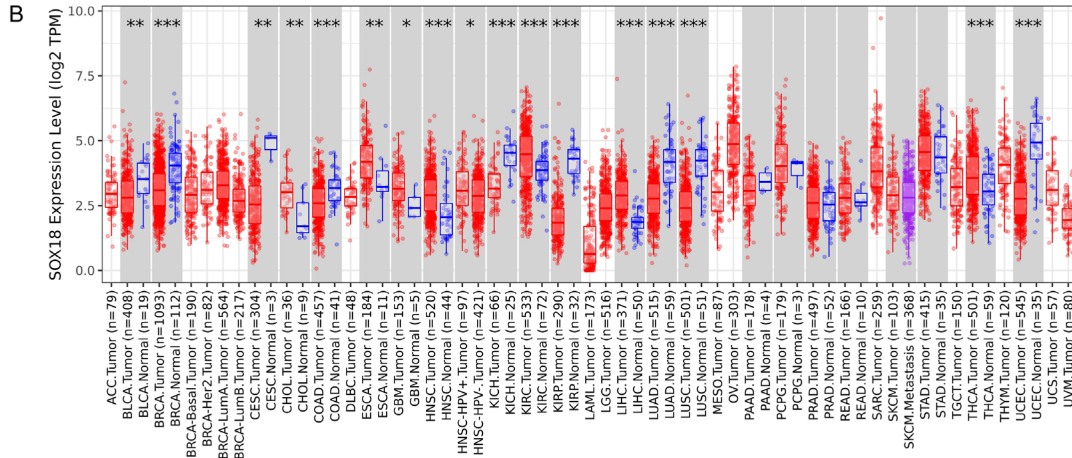
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17


B
SOX18 Expression Level (log2 TPM)
10.0
7.5
5.0
2.5
0.0
ACC.Tumor (n=79)
BLCA.Tumor (n=408)
BLCA.Normal (n=19)
BRCA.Tumor (n=1093)
BRCA.Normal (n=112)
BRCA-Basal.Tumor (n=190)
BRCA-Her2.Tumor (n=82)
BRCA-LumA.Tumor (n=564)
BRCA-LumB.Tumor (n=217)
CESC.Tumor (n=304)
CESC.Normal (n=3)
CHOL.Tumor (n=36)
CHOL.Normal (n=9)
COAD.Tumor (n=457)
COAD.Normal (n=41)
DLBC.Tumor (n=48)
ESCA.Tumor (n=184)
ESCA.Normal (n=11)
GBM.Tumor (n=153)
GBM.Normal (n=5)
HNSC.Tumor (n=520)
HNSC.Normal (n=44)
HNSC-HPV+.Tumor (n=97)
HNSC-HPV-.Tumor (n=421)
KICH.Tumor (n=66)
KICH.Normal (n=25)
KIRC.Tumor (n=533)
KIRC.Normal (n=72)
KIRP.Tumor (n=290)
KIRP.Normal (n=32)
LAML.Tumor (n=173)
LGG.Tumor (n=516)
LIHC.Tumor (n=371)
LIHC.Normal (n=50)
LUAD.Tumor (n=515)
LUAD.Normal (n=59)
LUSC.Tumor (n=501)
LUSC.Normal (n=51)
MESO.Tumor (n=87)
OV.Tumor (n=303)
PAAD.Tumor (n=178)
PAAD.Normal (n=4)
PCPG.Tumor (n=179)
PCPG.Normal (n=3)
PRAD.Tumor (n=497)
PRAD.Normal (n=52)
READ.Tumor (n=166)
READ.Normal (n=10)
SARC.Tumor (n=259)
SKCM.Tumor (n=103)
SKCM.Metastasis (n=368)
STAD.Tumor (n=415)
STAD.Normal (n=35)
TGCT.Tumor (n=150)
THCA.Tumor (n=501)
THCA.Normal (n=59)
THYM.Tumor (n=120)
UCEC.Tumor (n=545)
UCEC.Normal (n=35)
UCS.Tumor (n=57)
UVM.Tumor (n=80)

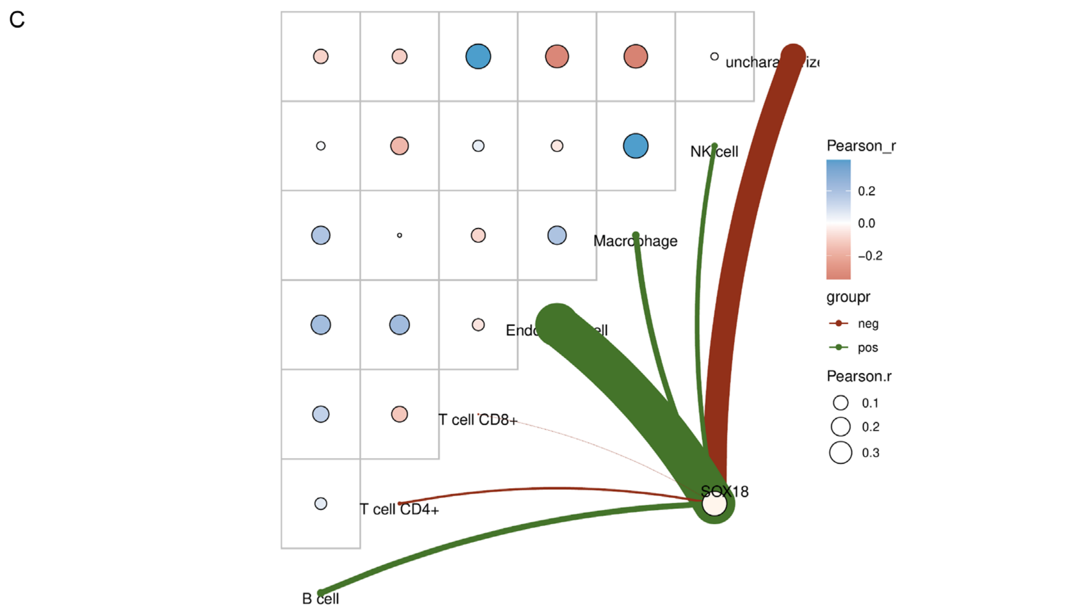

C
NK cell
Macrophage
T cell CD8+
T cell CD4+
B cell
SOX18
Pearson_r
0.2
0.0
−0.2
groupr
neg
pos
Pearson.r
0.1
0.2
0.3

Figure 1. Analysis of SOX18 expression in head and neck cancer and its implications for patient survival and tumor immunity. A. Kaplan-Meier survival curves for head and neck cancer patients stratified by SOX18 expression levels. Patients with high SOX18 expression (blue line) exhibit a significantly higher 5-year survival rate (55%) compared to those with low SOX18 expression (red line), who have a 5-year survival rate of 40%. Statistical analysis was performed using the log-rank test, indicating significant differences between the two groups. B. Comparative analysis of SOX18 expression in tumor tissues versus adjacent normal tissues across various cancer types. The data reveal a pronounced overexpression of SOX18 in tumor tissues compared to normal tissues, particularly in head and neck cancer. The box plots illustrate the median, interquartile range, and potential outliers for SOX18 expression levels, emphasizing significant differences (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). C. Correlation analysis between SOX18 expression and tumor endothelial cells in head and neck cancer. A significant negative correlation (Spearman's rho = -0.45, $P < 0.01$) was observed, suggesting that higher SOX18 expression is associated with lower levels of tumor endothelial cells. This relationship underscores the potential role of SOX18 in modulating tumor angiogenesis and immune evasion within the tumor microenvironment. Data from TCGA (https://portal.gdc.cancer.gov/).

Further investigation into the relationship between SOX18 expression and immune responses within tumors revealed a marked inverse correlation between SOX18 expression and the presence of tumor endothelial cells, as detailed in **Figure 1C**. This insight could offer a deeper understanding of SOX18's potential role in tumor angiogenesis and immune evasion. These findings not only enhance our understanding of SOX18's role in head and neck cancer but also suggest its potential as a prognostic biomarker and therapeutic target in cancer treatment.

SOX18 silencing reduced proliferation in NPC cells

We examined SOX18 expression in the NP-69 normal cell line and three NPC cell lines: C666-1, HK-1, and 5-8f, as illustrated in **Figure 2B**. The comparative analysis revealed that the SOX18 expression level in the 5-8f cell line was approximately four-fold higher than that observed in the NP-69 normal cell line. Similarly, as shown in **Figure 2A**, we found that the expression level of SOX18 in NPC tissues was approximately 4-fold higher than that in paraneoplastic tissues. To further explore the role of SOX18, we silenced its expression using small interfering RNA (siRNA) in the 5-8f cell line. This approach successfully reduced SOX18 expression to approximately 25% of its initial level, as depicted in **Figure 2C**. Additionally, Western blot analysis confirmed that SOX18 levels were decreased by nearly 50%, as shown in **Figure 2D**.

To assess the effects of SOX18 silencing on cellular proliferation, we compared the proliferation rates in the si-SOX18 treated group against the control group using CCK-8 assay, as presented in **Figure 2E**. The results revealed a marked decrease in the proliferation of 5-8f cells following SOX18 silencing. These results underscore the pivotal function of SOX18 in regulating NPC cell proliferation and suggest that targeting SOX18 may offer a promising therapeutic strategy for cancer treatment.

SOX18 silencing increased apoptosis and reduced colony formation

In this study, cell apoptosis was assessed using flow cytometry. The comparison between the SOX18 silenced group and the control group revealed that the suppression of SOX-18 expression led to a significant increase in apoptosis, with a rise of over 5%, which was statistically significant, as shown in **Figure 3A**. Additionally, a colony formation assay was conducted to evaluate the clonal expansion capacity of the cells. The outcomes indicated that the number of colonies formed by the cells with silenced SOX18 was significantly diminished by nearly 30% compared to the control group, as depicted in **Figure 3B**. These results underscore the vital role of SOX18 in promoting cellular survival and proliferation.

SOX18 silencing reduced cell invasion and migration

To assess the invasive properties of the cells, a Transwell invasion assay was performed. The results revealed that SOX18 silencing significantly decreased the invasiveness, with an approximately 30% reduction in the number of cells that invaded through the membrane, as shown in **Figure 4A**. In addition, a wound healing assay was conducted to assess cell migratory rate. The result indicated that SOX18-silenced cells migrated a significantly shorter

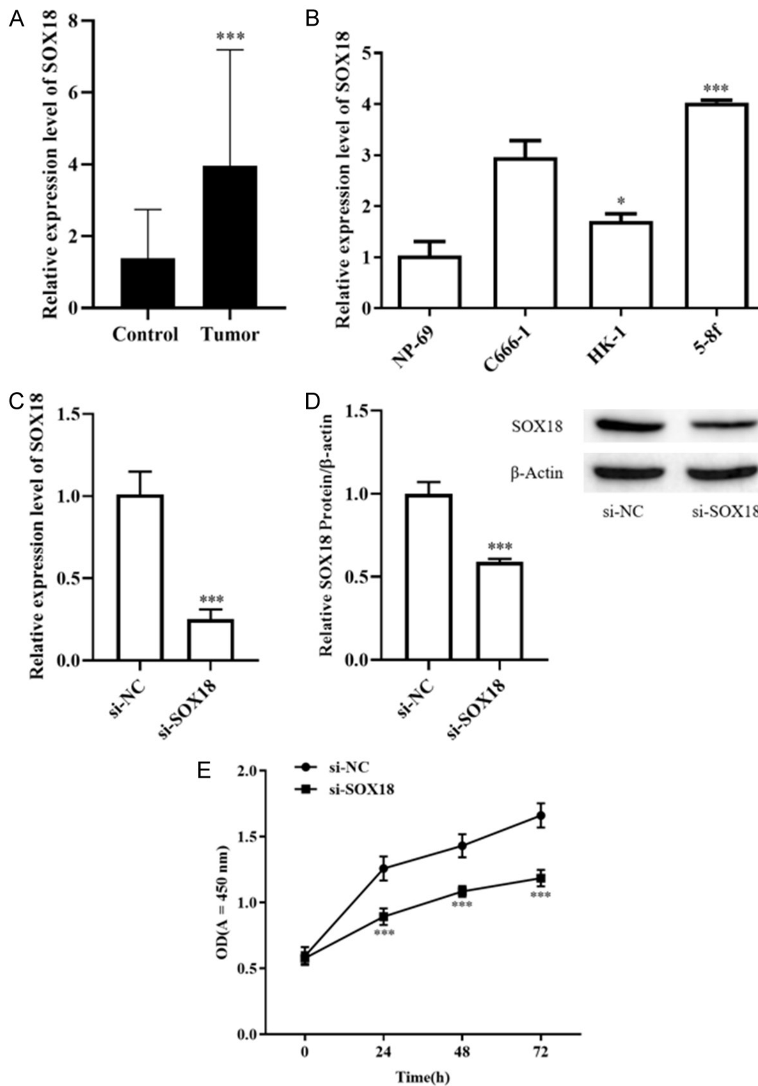


Figure 2. SOX18 expression and its effect on proliferation in NPC cells. A. SOX18 mRNA expression levels in tumor (Nasopharyngeal cancer tumor tissue) and control (paraneoplastic tissue), showing that tumor tissue has approximately four times higher SOX18 expression compared to control. B. Quantitative comparison of SOX18 mRNA expression levels, showing that 5-8f cells have approximately four times higher SOX18 expression compared to NP-69 cells. Statistical significance was determined using a t-test ($P < 0.01$). C. SOX18 mRNA expression in 5-8f cells after siRNA-mediated silencing (si-SOX18) compared to control (si-Control). Silencing reduced SOX18 expression to approximately 25% of the original level. D. Western blot analysis of SOX18 protein levels in 5-8f cells post-siRNA treatment, showing a reduction to approximately half of the original protein levels. E. Cell proliferation assessed by CCK-8 assay in 5-8f cells after SOX18 silencing, indicating a significant reduction in cell proliferation (*$P < 0.05$ and ***$P < 0.001$).

distance, with a nearly 30% reduction in migration, as shown in **Figure 4B**. Collectively, these observations emphasize the essential role of SOX18 in regulating cell invasion and migration, proposing that targeting SOX18 may be a promising strategy for NPC treatment.

SOX18 silencing downregulated Wnt/β-catenin pathway genes and protein

To investigate the impact of SOX18 silencing on the Wnt/β-catenin signaling pathway, RT-PCR was performed to quantify the mRNA levels of C-myc, β-catenin, and TCF4. The data revealed a notable decrease in the expression of these genes in the SOX18-silenced group, as shown in **Figure 5A**. Additionally, Western blot analysis was utilized to evaluate the protein levels of C-myc, β-catenin, TCF4, and phosphorylated glycogen synthase kinase 3 beta (p-GSK3β), as depicted in **Figure 5B**. The results indicated a significant reduction in the protein levels of these markers in the SOX18-silenced group.

Discussion

SOX18, as a key transcription factor, plays an important role in the genesis and development of multiple cancers. Recent studies have shown that SOX18 is involved in various physiological processes, including angiogenesis, cell proliferation, migration and differentiation. Additionally, it contributes to tumor development. However, in nasopharyngeal carcinoma (NPC), a special tumor of epithelial origin, the expression of SOX18 and its mechanism of action have not been thoroughly explored. This study aims to explore the role of SOX18 in NPC by investigating its cellular functions through gene knockdown and signaling pathway analysis. The goal is to assess its association with various tumor biological features and provide a theoretical basis for potential future diagnostic and therapeutic strategies.

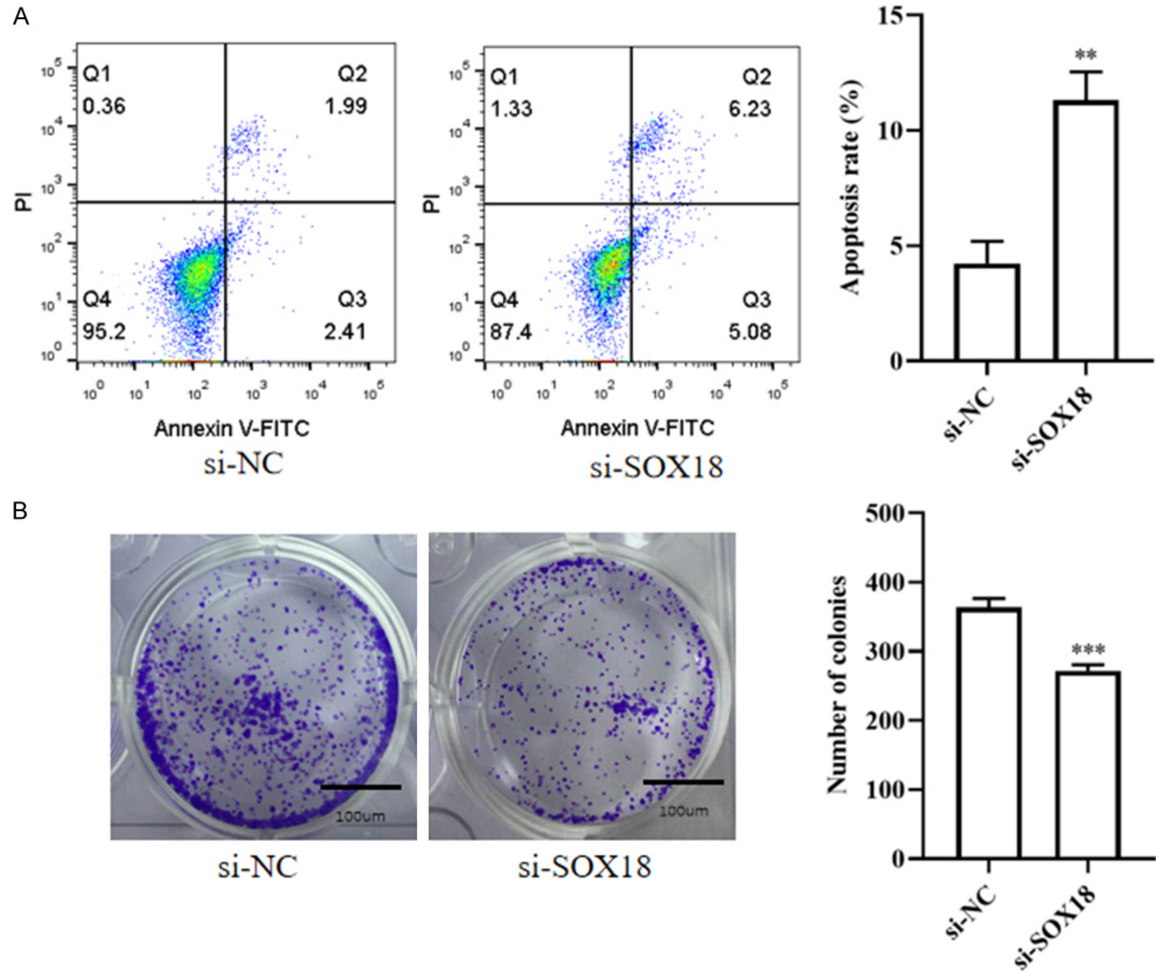


Figure 3. Effects of SOX18 silencing on apoptosis and colony formation in NPC cells. A. Apoptosis rates in 5-8f cells measured by flow cytometry using Annexin V/PI staining. Silencing SOX18 resulted in a significant increase in apoptosis by over 5% compared to the control group. B. Colony formation assay showing the number of colonies formed by SOX18-silenced 5-8f cells compared to control. There was a significant reduction in colony formation by approximately 30% in the SOX18-silenced group (**P < 0.01 and ***P < 0.001).

We observed a significant upregulation of SOX18 expression in NPC cell lines compared to normal cells, underscoring its potential involvement in tumor progression. Functional assays demonstrated that SOX18 silencing markedly inhibited NPC cell proliferation, migration, and invasion, while promoting apoptosis and reducing colony formation. These findings align with observations in other cancers [13, 15, 16, 32, 47, 48], such as laryngeal cancer [13] and hepatocellular carcinoma [16], where elevated SOX18 expression correlates with increased proliferation and suppressed apoptosis. Further analysis using CCK-8 assays confirmed that SOX18 silencing significantly attenuated NPC cell proliferation. Transwell invasion and scratch wound healing assays substantiated the reduction in invasion and migration capacities following SOX18 knockdown. Collectively, these results underscore the pivotal role of SOX18 in driving the invasive phenotype of NPC and highlight its involvement in NPC progression by modulating cell growth and metastatic potential.

Tumor angiogenesis is critical for tumor growth and metastasis [49-52], and SOX18 plays an important regulatory role in this process [21, 28, 53]. Our findings indicate that SOX18 is negatively correlated with the expression of tumor endothelial cells, suggesting that SOX18 may influence tumor growth and metastasis by regulating angiogenesis in the tumor microenvironment. This finding is consistent with a cervi-

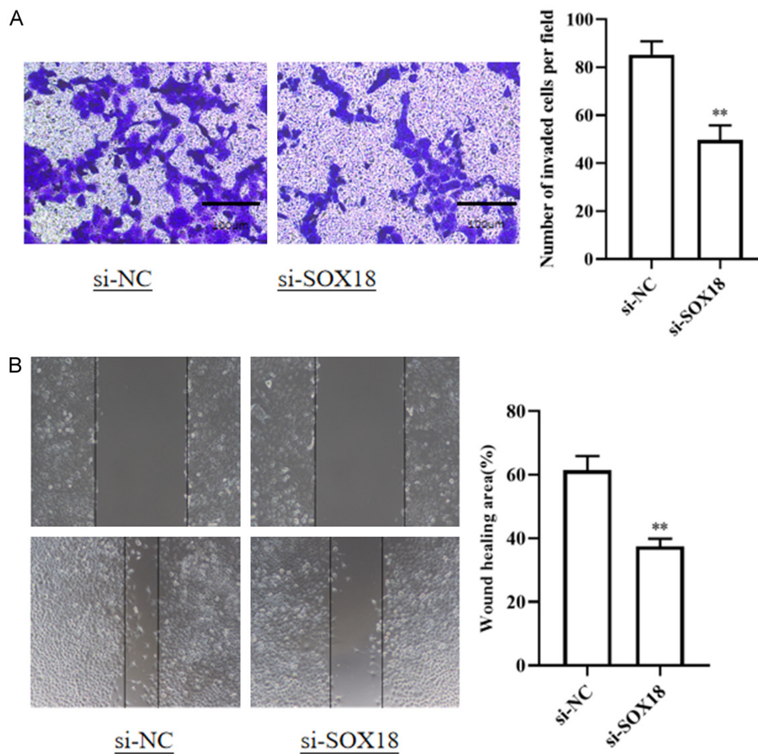


Figure 4. Impact of SOX18 silencing on cell invasion and migration in NPC cells. A. Transwell invasion assay results for 5-8f cells post-SOX18 silencing, showing a significant reduction in the number of invading cells by approximately 30% compared to control. B. Wound healing assay results demonstrating reduced migration distance in SOX18-silenced 5-8f cells by approximately 30% (**$P < 0.01$).

cal cancer study in which SOX18 served as a novel target gene for Hedgehog signaling, promoting cell migration and invasion [23]. Therefore, targeted inhibition of SOX18 may effectively block tumor angiogenesis, thereby hindering tumor growth and metastasis.

In this study, we also discovered that SOX18 modulates NPC cell behavior through the Wnt/β-catenin signaling pathway. Silencing SOX18 resulted in a significant downregulation of key genes and proteins in the Wnt/β-catenin pathway, such as C-myc, β-catenin, TCF4, and phosphorylated GSK3β. These findings suggest that SOX18 may influence tumor cell proliferation, migration, and invasion by modulating Wnt/β-catenin pathway activity [54, 55]. The Wnt/β-catenin pathway is crucial for the development and progression of various cancers [56-58], with aberrant activation often associated with invasiveness and poor survival rates [59, 60]. Our findings provide new insights into the specific mechanisms of SOX18 in tumors and lay a theoretical foundation for developing SOX18-targeted therapies.

The expression level of SOX18 is closely associated with the stage, grade and prognosis of various cancers [10, 29, 30, 48, 61, 62]. In a survival analysis of clinical data, analysis of head and neck and nasopharyngeal cancer data from the Human Protein Atlas (HPA) database confirmed that high SOX18 expression was associated with poorer prognosis. This result is consistent with the correlation of high SOX18 expression with advanced stage and poor prognosis in ovarian and hepatocellular carcinomas [16, 31]. Therefore, SOX18 holds promise as a prognostic biomarker for NPC, helping to develop a personalized treatment plan.

Our results suggest that SOX18 is an important regulator of tumor cell proliferation and survival, making it a promising therapeutic target. Targeting SOX18 by small molecule inhibitors or siRNA-based approaches may provide new avenues for NPC treatment. Inhibiting SOX18 expression or function not only suppresses tumor growth and metastasis but also enhances the sensitivity of tumors to existing therapies, improving patient prognosis and providing new treatment options for cancer patients [1, 15, 19, 47, 63, 64]. Currently, targeted therapeutic strategy is still in the exploratory stage, and future therapeutic strategies could focus on the development of small molecule inhibitors [34-36, 65, 66], antibodies [37], or gene therapies targeting [38] that specifically and efficiently target SOX18. Such strategies could inhibit SOX18 activity, block its associated signaling pathways, and disrupt the cellular processes it regulates, ultimately halting or even reversing tumor progression. In addition, combining SOX18-targeted therapies with other treatments, such as targeted thera-

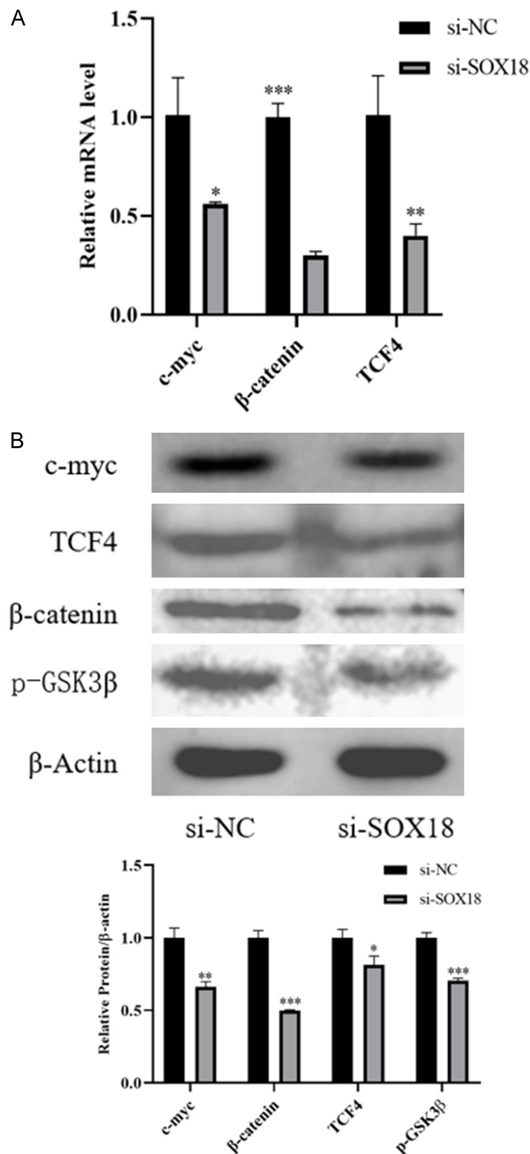


Figure 5. Effect of SOX18 silencing on Wnt/β-catenin pathway related genes and proteins in NPC cells. A. RT-PCR analysis for mRNA levels of C-myc, β-catenin, and TCF4 in 5-8f cells after SOX18 silencing, showing significant reductions compared to control. B. Western blot analysis for protein levels of C-myc, β-catenin, TCF4, and phosphorylated GSK3β (p-GSK3β) in 5-8f cells post-SOX18 silencing, showing a significant decrease in the protein levels of these factors (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

pies or immunotherapies, may enhance therapeutic effects and provide more effective treatment options for NPC patients.

Although this study highlights the significant role and potential mechanisms of SOX18 in NPC, further research is needed to fully understand its specific role within the complex tumorigenesis and progression network. Future studies should include larger clinical samples and multicenter data analysis to validate the practical application of SOX18 as a prognostic biomarker and therapeutic target. In addition, exploring the interactions between SOX18 and other key signaling pathways, such as PI3K/AKT [67] and MAPK [68, 69], could provide deeper insights into its comprehensive role in tumors.

While SOX18 is classically recognized as a transcription factor, it may also be involved in tumor progression through non-transcriptional mechanisms. For example, SOX18 may regulate a variety of biological behaviors in tumor cells by interacting with other transcription factors and epigenetic modifiers. Therefore, future studies should explore its role within the tumor microenvironment, especially its potential impact on tumor immune escape and drug resistance, potentially revealing its multifaceted role in cancer biology.

In summary, through systematic experiments and data analysis, this study reveals the crucial role and potential mechanisms of SOX18 in NPC, providing new scientific evidence for its potential as a diagnostic and therapeutic target. Future research should continue to explore the specific functions and mechanisms of SOX18 in tumors, offering more precise theoretical support and application prospects for personalized cancer therapy.

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Disclosure of conflict of interest

None.

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References

[1] Grimm D, Bauer J, Wise P, Kruger M, Simonsen U, Wehland M, Infanger M and Corydon TJ. The role of SOX family members in solid tumours and metastasis. Semin Cancer Biol 2020; 67: 122-153.

[2] Abadi AJ, Zarrabi A, Hashemi F, Zabolian A, Najafi M, Entezari M, Hushmandi K, Aref AR, Khan H, Makvandi P, Ashrafizaveh S, Farkhondeh T, Ashrafizadeh M, Samarghandian S and Hamblin MR. The role of SOX family transcription factors in gastric cancer. Int J Biol Macromol 2021; 180: 608-624.

[3] Treccarichi S, Cali F, Vinci M, Ragalmuto A, Musumeci A, Federico C, Costanza C, Bottitta M, Greco D, Saccone S and Elia M. Implications of a de novo variant in the SOX12 gene in a patient with generalized epilepsy, intellectual disability, and childhood emotional behavioral disorders. Curr Issues Mol Biol 2024; 46: 6407-6422.

[4] Zheng H, Liu M, Shi S, Huang H, Yang X, Luo Z, Song Y, Xu Q, Li T, Xue L, Lu F and Wang J. MAP4K4 and WT1 mediate SOX6-induced cellular senescence by synergistically activating the ATF2-TGFbeta2-Smad2/3 signaling pathway in cervical cancer. Mol Oncol 2024; 18: 1327-1346.

[5] Del Puerto HL, Miranda APGS, Qutob D, Ferreira E, Silva FHS, Lima BM, Carvalho BA, Roque-Souza B, Gutseit E, Castro DC, Pozzolini ET, Duarte NO, Lopes TBG, Taborda DYO, Quirino SM, Elgerbi A, Choy JS and Underwood A. Clinical correlation of transcription factor SOX3 in cancer: unveiling its role in tumorigenesis. Genes (Basel) 2024; 15: 777.

[6] Jiang J, Wang Y, Sun M, Luo X, Zhang Z, Wang Y, Li S, Hu D, Zhang J, Wu Z, Chen X, Zhang B, Xu X, Wang S, Xu S, Huang W and Xia L. SOX on tumors, a comfort or a constraint? Cell Death Discov 2024; 10: 67.

[7] Kumar P and Mistri TK. Transcription factors in SOX family: potent regulators for cancer initiation and development in the human body. Semin Cancer Biol 2020; 67: 105-113.

[8] Higashijima Y and Kanki Y. Molecular mechanistic insights: the emerging role of SOXF transcription factors in tumorigenesis and development. Semin Cancer Biol 2020; 67: 39-48.

[9] Chen J, Feng W, Sun M, Huang W, Wang G, Chen X, Yin Y, Chen X, Zhang B, Nie Y, Fan D, Wu K and Xia L. TGF-beta1-induced SOX18 elevation promotes hepatocellular carcinoma progression and metastasis through transcriptionally upregulating PD-L1 and CXCL12. Gastroenterology 2024; 167: 264-280.

[10] Neinaa YME, El-Ashmawy AA, Alshenawy HA and Arakeeb EEA. Significance of SOX18 expression in nonmelanoma skin cancers for prediction of high-risk patients: a preliminary study. Int J Dermatol 2020; 59: 1117-1124.

[11] Zhang C, Chen Z, Gao N, Xiong G, Chen P, Li H, Chen D, He Q and Peng L. SOX18 meditates the resistance of Bmi1-expressing cells to cetuximab in HNSCC. Oral Dis 2024; 30: 1100-1113.

[12] Rubannelsonkumar C, Ojile J, Mendez J, Valle D and Shackleford T. Role of SOX18 in promoting tumorigenesis in pediatric cancer cell lines. FASEB J 2022; 36.

[13] Xu Y, Zhang Q, Zhou J, Li Z, Guo J, Wang W and Wang W. Down-regulation of SOX18 inhibits laryngeal carcinoma cell proliferation, migration, and invasion through JAK2/STAT3 signaling. Biosci Rep 2019; 39: BSR20182480.

[14] Miao Z, Deng X, Shuai P and Zeng J. Upregulation of SOX18 in colorectal cancer cells promotes proliferation and correlates with colorectal cancer risk. Onco Targets Ther 2018; 11: 8481-8490.

[15] Zhu D, Yang D, Li X and Feng F. Heterogeneous expression and biological function of SOX18 in osteosaroma. J Cell Biochem 2018; 119: 4184-4192.

[16] Wang G, Wei Z, Jia H, Zhao W, Yang G and Zhao H. Knockdown of SOX18 inhibits the proliferation, migration and invasion of hepatocellular carcinoma cells. Oncol Rep 2015; 34: 1121-1128.

[17] Wang L, Zhang Q, Wu P, Xiang W, Xie D, Wang N, Deng M, Cao K, Zeng H, Xu Z, Xiaoming Liu, He L, Long Z, Tan J, Wang J, Liu B and Liu J. SLC12A5 interacts and enhances SOX18 activity to promote bladder urothelial carcinoma progression via upregulating MMP7. Cancer Sci 2020; 111: 2349-2360.

[18] Rosati D, Palmieri M, Brunelli G, Morrione A, Iannelli F, Frullanti E and Giordano A. Differential gene expression analysis pipelines and bioinformatic tools for the identification of specific biomarkers: a review. Comput Struct Biotechnol J 2024; 23: 1154-1168.

[19] Huaqi Y, Caipeng Q, Qiang W, Yiqing D and Tao X. The role of SOX18 in bladder cancer and its underlying mechanism in mediating cellular functions. Life Sci 2019; 232: 116614.

[20] Wang Y and Kanneganti TD. From pyroptosis, apoptosis and necroptosis to PANoptosis: a mechanistic compendium of programmed cell death pathways. Comput Struct Biotechnol J 2021; 19: 4641-4657.

[21] Young N, Hahn CN, Poh A, Dong C, Wilhelm D, Olsson J, Muscat GE, Parsons P, Gamble JR and Koopman P. Effect of disrupted SOX18 transcription factor function on tumor growth, vascularization, and endothelial development. J Natl Cancer Inst 2006; 98: 1060-1067.

[22] Jiang X, Wang J, Deng X, Xiong F, Zhang S, Gong Z, Li X, Cao K, Deng H, He Y, Liao Q, Xiang B, Zhou M, Guo C, Zeng Z, Li G, Li X and Xiong W. The role of microenvironment in tumor angiogenesis. J Exp Clin Cancer Res 2020; 39: 204.

[23] Petrovic I, Milivojevic M, Popovic J, Schwirtlich M, Rankovic B and Stevanovic M. SOX18 is a novel target gene of hedgehog signaling in cervical carcinoma cell lines. PLoS One 2015; 10: e0143591.

[24] Guo H, Hu Z, Yang X, Yuan Z, Wang M, Chen C, Xie L, Gao Y, Li W, Bai Y and Lin C. Smad4 regulates TGF-beta1-mediated Hedgehog activation to promote epithelial-to-mesenchymal transition in pancreatic cancer cells by suppressing Gli1 activity. Comput Struct Biotechnol J 2024; 23: 1189-1200.

[25] Skoda AM, Simovic D, Karin V, Kardum V, Vranic S and Serman L. The role of the Hedgehog signaling pathway in cancer: a comprehensive review. Bosn J Basic Med Sci 2018; 18: 8-20.

[26] Villani R, Sim SL, Roy E, Wainwright B, Francois M and Khosrotehrani K. Ectopic expression of SOX18 in Basal cell carcinoma negatively regulates tumour progression. J Dermatol Sci 2020; 98: 179-185.

[27] Yang H, Lin H and Sun X. Multiscale modeling of drug resistance in glioblastoma with gene mutations and angiogenesis. Comput Struct Biotechnol J 2023; 21: 5285-5295.

[28] Ayoub NM, Jaradat SK, Al-Shami KM and Alkhalifa AE. Targeting angiogenesis in breast cancer: current evidence and future perspectives of novel anti-angiogenic approaches. Front Pharmacol 2022; 13: 838133.

[29] Yin H, Sheng Z, Zhang X, Du Y, Qin C, Liu H, Dun Y, Wang Q, Jin C, Zhao Y and Xu T. Overexpression of SOX18 promotes prostate cancer progression via the regulation of TCF1, c-Myc, cyclin D1 and MMP-7. Oncol Rep 2017; 37: 1045-1051.

[30] Qin S, Liu G, Jin H, Chen X, He J, Xiao J, Qin Y, Mao Y and Zhao L. The dysregulation of SOX family correlates with DNA methylation and immune microenvironment characteristics to predict prognosis in hepatocellular carcinoma. Dis Markers 2022; 2022: 2676114.

[31] Pula B, Kobierzycki C, Solinski D, Olbromski M, Nowak-Markwitz E, Spaczynski M, Kedzia W, Zabel M and Dziegiel P. SOX18 expression predicts response to platinum-based chemotherapy in ovarian cancer. Anticancer Res 2014; 34: 4029-4037.

[32] Sun Y, Lei B and Huang Q. SOX18 affects cell viability, migration, invasiveness, and apoptosis in hepatocellular carcinoma (HCC) cells by participating in epithelial-to-mesenchymal transition (EMT) progression and adenosine monophosphate activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR). Med Sci Monit 2019; 25: 6244-6254.

[33] Chen J, Du F, Dang Y, Li X, Qian M, Feng W, Qiao C, Fan D, Nie Y, Wu K and Xia L. Fibroblast growth factor 19-mediated up-regulation of SYR-related high-mobility group box 18 promotes hepatocellular carcinoma metastasis by transactivating fibroblast growth factor receptor 4 and Fms-related tyrosine kinase 4. Hepatology 2020; 71: 1712-1731.

[34] Overman J, Fontaine F, Moustaqil M, Mittal D, Sierecki E, Sacilotto N, Zuegg J, Robertson AAB, Holmes K, Salim AA, Mamidyala S, Butler MS, Robinson AS, Lesieur E, Johnston W, Alexandrov K, Black BL, Hogan BM, De Val S, Capon RJ, Carroll JS, Bailey TL, Koopman P, Jauch R, Cooper MA, Gambin Y and Francois M. Pharmacological targeting of the transcription factor SOX18 delays breast cancer in mice. Elife 2017; 6: e21221.

[35] Fontaine F, Overman J, Moustaqil M, Mamidyala S, Salim A, Narasimhan K, Prokoph N, Robertson AAB, Lua L, Alexandrov K, Koopman P, Capon RJ, Sierecki E, Gambin Y, Jauch R, Cooper MA, Zuegg J and Francois M. Small-molecule inhibitors of the SOX18 transcription factor. Cell Chem Biol 2017; 24: 346-359.

[36] Zhu W, Wang Y, Zhang D, Yu X and Leng X. MiR-7-5p functions as a tumor suppressor by targeting SOX18 in pancreatic ductal adenocarcinoma. Biochem Biophys Res Commun 2018; 497: 963-970.

[37] Fontaine FR, Goodall S, Prokop JW, Howard CB, Moustaqil M, Kumble S, Rasicci DT, Osborne GW, Gambin Y, Sierecki E, Jones ML, Zuegg J, Mahler S and Francois M. Functional domain analysis of SOX18 transcription factor using a single-chain variable fragment-based approach. MAbs 2018; 10: 596-606.

[38] Klaus M, Prokoph N, Girbig M, Wang X, Huang YH, Srivastava Y, Hou L, Narasimhan K, Kolatkar PR, Francois M and Jauch R. Structure and decoy-mediated inhibition of the SOX18/Prox1-DNA interaction. Nucleic Acids Res 2016; 44: 3922-3935.

[39] Lin Y, Huang Y, Yang B, Zhang Y, Ji N, Li J, Zhou Y, Shen YQ and Chen Q. Precision therapy targeting CAMK2 to overcome resistance to EGFR inhibitors in FAT1-mutated oral squamous cell carcinoma. Chin Med J (Engl) 2024; [Epub ahead of print].

[40] Huang H, Yao Y, Deng X, Huang Z, Chen Y, Wang Z, Hong H, Huang H and Lin T. Immunotherapy for nasopharyngeal carcinoma: current status and prospects (Review). Int J Oncol 2023; 63: 97.
[41] Zheng M, Ren Y, Jing L, Cheng M, Lin J and Yu Y. Nasopharyngeal carcinoma cell screening based on nuclear targeting Surface-Enhanced Raman Scattering (SERS) detection. Anal Chim Acta 2024; 1316: 342864.
[42] Xiao B, Zhang W, Kuang Z, Lu J, Li W, Deng C, He Y, Lei T, Hao W, Sun Z and Li L. SOX9 promotes nasopharyngeal carcinoma cell proliferation, migration and invasion through BMP2 and mTOR signaling. Gene 2019; 715: 144017.
[43] Zhang S, Li S and Gao JL. Promoter methylation status of the tumor suppressor gene SOX11 is associated with cell growth and invasion in nasopharyngeal carcinoma. Cancer Cell Int 2013; 13: 109.
[44] Huang DY, Lin YT, Jan PS, Hwang YC, Liang ST, Peng Y, Huang CY, Wu HC and Lin CT. Transcription factor SOX-5 enhances nasopharyngeal carcinoma progression by down-regulating SPARC gene expression. J Pathol 2008; 214: 445-455.
[45] Dong DN, Fan PW, Feng YN, Liu GH, Peng YC, Dong T, Wang RZ and Yu JM. Association between circulating CD39+CD8+ T cells prechemoradiotherapy and prognosis in patients with nasopharyngeal carcinoma. Chin Med J (Engl) 2021; 134: 2066-2072.
[46] Olbromski M, Podhorska-Okolow M and Dziegiel P. Role of the SOX18 protein in neoplastic processes. Oncol Lett 2018; 16: 1383-1389.
[47] Huaqi Y, Caipeng Q, Qiang W, Yiqing D, Xiang D, Xu T, Xiaowei Z, Qing L, Shijun L and Tao X. Transcription factor SOX18 promotes clear cell renal cell carcinoma progression and alleviates cabozantinib-mediated inhibitory effects. Mol Cancer Ther 2019; 18: 2433-2445.
[48] Wang Y, Guo H, Zhang D, Yu X, Leng X, Li S and Zhu W. Overexpression of SOX18 correlates with accelerated cell growth and poor prognosis in human pancreatic ductal adenocarcinoma. Biochem Biophys Res Commun 2016; 479: 510-516.
[49] Leite de Oliveira R, Hamm A and Mazzone M. Growing tumor vessels: more than one way to skin a cat - implications for angiogenesis targeted cancer therapies. Mol Aspects Med 2011; 32: 71-87.
[50] Shukla NA, Yan MN and Hanna N. The story of angiogenesis inhibitors in non-small-cell lung cancer: the past, present, and future. Clin Lung Cancer 2020; 21: 308-313.
[51] Zhang J, Lu T, Lu S, Ma S, Han D, Zhang K, Xu C, Liu S, Gan L, Wu X, Yang F, Wen W and Qin W. Single-cell analysis of multiple cancer types reveals differences in endothelial cells between tumors and normal tissues. Comput Struct Biotechnol J 2022; 21: 665-676.
[52] Helfinger V, Henke N, Harenkamp S, Walter M, Epah J, Penski C, Mittelbronn M and Schroder K. The NADPH Oxidase Nox4 mediates tumour angiogenesis. Acta Physiol (Oxf) 2016; 216: 435-446.
[53] Duong T, Koltowska K, Pichol-Thievend C, Le Guen L, Fontaine F, Smith KA, Truong V, Skoczylas R, Stacker SA, Achen MG, Koopman P, Hogan BM and Francois M. VEGFD regulates blood vascular development by modulating SOX18 activity. Blood 2014; 123: 1102-1112.
[54] Balatskyi VV, Sowka A, Dobrzyn P and Piven OO. WNT/beta-catenin pathway is a key regulator of cardiac function and energetic metabolism. Acta Physiol (Oxf) 2023; 237: e13912.
[55] Alonso-Martin S, Aurade F, Mademtzoglou D, Rochat A, Zammit PS and Relaix F. SOXF factors regulate murine satellite cell self-renewal and function through inhibition of beta-catenin activity. Elife 2018; 7: e26039.
[56] Geng Q, Deng H, Fu J and Cui F. SOX18 exerts tumor-suppressive functions in papillary thyroid carcinoma through inhibition of Wnt/beta-catenin signaling. Exp Cell Res 2020; 396: 112249.
[57] Klaus A and Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008; 8: 387-398.
[58] Zhao H, Gong H, Zhu P, Sun C, Sun W, Zhou Y, Wu X, Qiu A, Wen X, Zhang J, Luo D, Liu Q and Li Y. Deciphering the cellular and molecular landscapes of Wnt/beta-catenin signaling in mouse embryonic kidney development. Comput Struct Biotechnol J 2024; 23: 3368-3378.
[59] Song P, Gao Z, Bao Y, Chen L, Huang Y, Liu Y, Dong Q and Wei X. Wnt/beta-catenin signaling pathway in carcinogenesis and cancer therapy. J Hematol Oncol 2024; 17: 46.
[60] Yoshida GJ. Regulation of heterogeneous cancer-associated fibroblasts: the molecular pathology of activated signaling pathways. J Exp Clin Cancer Res 2020; 39: 112.
[61] Olbromski M, Podhorska-Okolow M and Dziegiel P. Role of SOX protein groups F and H in lung cancer progression. Cancers (Basel) 2020; 12: 3235.
[62] Jethon A, Pula B, Olbromski M, Werynska B, Muszczynska-Bernhard B, Witkiewicz W, Dziegiel P and Podhorska-Okolow M. Prognostic significance of SOX18 expression in non-small cell lung cancer. Int J Oncol 2015; 46: 123-132.
[63] Chen J, Dang Y, Feng W, Qiao C, Liu D, Zhang T, Wang Y, Tian D, Fan D, Nie Y, Wu K and Xia L. SOX18 promotes gastric cancer metastasis

through transactivating MCAM and CCL7. Oncogene 2020; 39: 5536-5552.

[64] Duong T, Proulx ST, Luciani P, Leroux JC, Detmar M, Koopman P and Francois M. Genetic ablation of SOX18 function suppresses tumor lymphangiogenesis and metastasis of melanoma in mice. Cancer Res 2012; 72: 3105-3114.

[65] Rodak O, Mrozowska M, Rusak A, Gomulkiewicz A, Piotrowska A, Olbromski M, Podhorska-Okolow M, Ugorski M and Dziegiel P. Targeting SOX18 transcription factor activity by small-molecule inhibitor Sm4 in non-small lung cancer cell lines. Int J Mol Sci 2023; 24: 11316.

[66] Cui Y, Man S, Tao J, Liu Y, Ma L, Guo L, Huang L, Liu C and Gao W. The lipid droplet in cancer: from being a tumor-supporting hallmark to clinical therapy. Acta Physiol (Oxf) 2024; 240: e14087.

[67] Liu T, Jin C, Sun J, Zhu L, Wang C, Xiao F, Liu X, Lv L, Yang X, Zhou W, Tan C, Wang X and Wei W. Paroxetine alleviates dendritic cell and T lymphocyte activation via GRK2-mediated PI3K-AKT signaling in rheumatoid arthritis. Chin Med J (Engl) 2024; [Epub ahead of print].

[68] Yu X, Qian F, Zhang X, Zhu Y, He G, Yang J, Wu X, Zhou Y, Shen L, Shi X, Zhang H and Liu X. Promotion effect of FOXCUT as a microRNA sponge for miR-24-3p on progression in triple-negative breast cancer through the p38 MAPK signaling pathway. Chin Med J (Engl) 2024; 137: 105-114.

[69] Yu ZY, Jiang XY, Zhao RR, Qin JJ, Luo CJ, Ren YX, Ren W, Ma ZJ and Jiao ZY. Effect of KIF22 on promoting proliferation and migration of gastric cancer cells via MAPK-ERK pathways. Chin Med J (Engl) 2020; 133: 919-928.