Heliyon 9 (2023) e22586

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

SOX8 promotes tumor growth and metastasis through FZD6-dependent Wnt/β-catenin signaling in colorectal carcinoma

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ARTICLE INFO

Keywords:
SOX8
FZD6
Metastasis
Wnt/β-catenin signaling
Colorectal carcinoma

ABSTRACT

SOX8 plays an important role in several physiological processes. Its expression is negatively associated with overall survival in patients with colorectal carcinoma (CRC), suggesting SOX8 is a potential prognostic factor for this disease. However, the role of SOX8 in CRC remains largely unknown. In this study, our data showed that *SOX8* expression was upregulated in CRC cell lines and tumor tissues. Stable knockdown of *SOX8* in CRC cell lines dramatically reduced cell proliferation, migration, and invasion. Furthermore, the knockdown of *SOX8* decreased the phospho-GSK3β level and suppressed Frizzled-6 (FZD6) transcription; restoration of *FZD6* expression partially abolished the effect of SOX8 on Wnt/β-catenin signaling and promote CRC cell proliferation. In conclusion, our findings suggested that SOX8 served as an oncogene in CRC through the activation of FZD6-dependent Wnt/β-catenin signaling.

1. Introduction

Colorectal carcinoma (CRC) is one of the most common types of cancer worldwide [1]. Over 50 % of CRC patients develop distant metastases, which is a major determinant of prognosis in this population [2,3]. Without clear symptoms at an early stage, less than 20 % of patients undergoing resection have optimal operative outcomes [4], suggesting the need to elucidate the molecular mechanisms underlying CRC development.

To date, more than 30 SOX family members mediating DNA binding through the highly conserved high-mobility group (HMG) domain. These proteins play important roles in embryonic development, cellular programming [5]. Their abnormal expression is involved in multiple human diseases, including cancer [6–8]. The SOX family are divided into eight groups: SOXA to SOXH [9]. Among them, SOX8, SOX9, and SOX10 are classified into the SOXE group [10,11]. Members of this group exert variable functions as either tumor suppressors or oncogenes in different human cancers. *SOX9* has been found to promote cell growth, migration, and invasion in thyroid cancer but inhibits melanoma development [12,13]. *SOX10* is overexpressed in bladder cancer and promotes malignancy of bladder cancer cells [14,15]. However, SOX10 stably silences the expression of *CMTM7* and inhibits the development of gastric cancer [15]. Notably, SOX9 plays oncogenic roles in cancer progression of CRC [16], whereas SOX10 acts as a tumor suppressor gene in this

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https://doi.org/10.1016/j.heliyon.2023.e22586
Received 24 May 2023; Received in revised form 5 November 2023; Accepted 15 November 2023
Available online 21 November 2023
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type of cancer [17].

SOX8 is upregulated in cisplatin-resistant tongue squamous cell carcinoma (TSCC) cells with CSC-like properties. In addition, SOX8 overexpression was highly correlated with lymph node metastasis, advanced tumor stage, and shorter overall survival. The mechanism is possibly due to SOX8-induced activation of the Wnt/β-catenin pathway. These results strongly suggest a correlation between SOX8 expression and clinical outcomes in patients with TSCC [18]. SOX8 is highly expressed in triple-negative breast cancer (TNBC) and acts as a functional oncogene by regulating tumorigenicity, migration, and apoptosis. Activation of the ZEB1-SOX8 axis predisposes TNBC to a relatively poor prognosis, which could be a promising therapeutic target for advanced TNBC [19]. In a retrospective study, SOX8 expression was found to be higher in CRC tissues than in adjacent non-tumor tissues. Clinically, high SOX8 expression is associated with poor overall survival (OS) after surgery in patients with CRC, suggesting its oncogenic features, while the underlying molecular mechanism remains unknown.

Wnt/β-catenin signaling participates in cancer development with different origins, particularly CRC. In CRC, Wnt/β-catenin signaling is involved in early progression, as well as invasion and metastasis in late stages. Additionally, overactivation of Wnt/β-catenin signaling promotes uncontrolled cell growth and prevents apoptosis [20]. To date, there are no Food and Drug Administration-approved Wnt signaling inhibitors for clinical use. Therefore, identifying additional information regarding Wnt signaling may provide a promising therapeutic strategy for CRC. Frizzled (FZD) family members are essential Wnt receptors. To date, 10 FZDs have been identified in humans. Wnt ligands bind to specific FZD and activate downstream Wnt signaling [21]. β-catenin translocates upon activation into the cell nucleus inducing the transcription of its target genes and regulating multiple cellular processes, such as cell proliferation and epithelial-to-mesenchymal transition (EMT) [22]. In conclusion, aberrant Wnt/β-catenin signaling can initiate uncontrolled proliferation and loss of epithelial differentiation, favoring tumor cell dissemination [23,24].

In the present study, we explored the function of SOX8 in CRC and demonstrated that *SOX8* expression knockdown leads to reduced cell proliferation, migration, and invasion. We also found that SOX8 regulates *FZD6* expression by binding to its promoter region and enhancing its transcriptional activity. Overall, we uncovered a novel mechanism in CRC and highlighted a key role of the SOX8/FZD6/Wnt/β-catenin axis in CRC development.

2. Materials and methods

2.1. Patients and tissue specimens

Fresh tumors and paired adjacent normal tissues were collected from 31 patients with CRC who underwent radical surgery at Peking University-Shenzhen Hospital between January 2020 and June 2020. Patients did not undergo chemotherapy or radiotherapy before surgery. All patients provided written informed consent to publish their images, clinical data, and other data included in our manuscript. This study was approved by the Institutional Ethics Committee of Shenzhen-Peking University-Hong Kong University of Science and Technology Medical Center (#2017–012).

2.2. 2D and 3D cell culture

Cell lines (Caco 2, HCT116, HT29, LoVo, SW480, and SW620) used in this study were purchased from the American Type Culture Collection (Manassas, VA, USA). All cells were regularly maintained in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Life Technologies) supplemented with 10 % fetal bovine serum (FBS; Gibco) and 1 % streptomycin and penicillin in a 37 °C incubator with 5 % CO_2.

For the generation of 3D spheroids, a basement membrane extract (BME)- based method was performed as described previously [25]. Briefly, a 96-well plate was coated with 50 μL of Matrigel (Corning) in each well at 37 °C for 1 h. Next, 1×10^4 cells were seeded in each well of a 96-well plate coated with Matrigel. Spheroids were photographed under a microscope (Leica, Germany) on day 7. The diameters of the spheroids were measured.

2.3. Plasmids and shRNA

The full-length Open Reading Frame (ORF) of *FZD6* was subcloned from pCMV6-XL5-FZD6 into the PCR4-TOPO plasmid (Invitrogen) with a His tag added to its N-terminus and a Flag tag added to its C-terminus. The sequence was verified using Sanger sequencing. PCR4-TOPO4-FZD6 was digested with *Bam*HI and *Eco*RI and ligated into the pcDNA3.1(+) expression vector. To construct cell lines with *SOX8* knockdown, HCT116 and SW620 cells were transfected with *SOX8* shRNA plasmids using Lipofectamine 3000. Geneticin (Roche Diagnostics) was used to select stable FZD6-expressing clones. Specific short hairpin RNAs (shRNAs) against *SOX8* were transduced into HCT116 and SW620 cell lines. The shRNA sequences for *SOX8* were as follows:5′-GCGCAGGAAGAGCGCCAAATT-3′ (shSOX8#1), 5′-GAUGAGUCUACUCGAGCAUTT-3′ (shSOX8#2), and 5′-CGUGUACAAGGCUGAAGCATT-3′ (shSOX8#3). Control cell lines were constructed by transduction of expression vector with scrambled shRNA.

2.4. Colony formation assay

HCT116 and SW620 cells with stable *SOX8* knockdown were seeded into 6-well plates. After day 7, colonies (≥50 cells/colony) were counted and stained with gentian violet. All experiments were performed in triplicates.

2.5. Cell proliferation assay

Cell Counting Kit-8 (CCK8; Dojindo Laboratories, Kumamoto, Japan) was used to evaluate cell viability. Briefly, HCT116 and SW620 cells with *SOX8* knockdown or matched negative controls were seeded in 96-well plates. Absorbance was measured at 450 nm at 24, 48, and 72 h, according to the manufacturer's instructions. Three experiments were performed in triplicates.

2.6. Wound healing, migration, and invasion assay

A scratch wound-healing assay was performed to evaluate cell motility. Briefly, HCT116 and SW620 cells were cultured in 6-well plates. Sterile tips were used to generate wounds in the cell layers. The wounded areas were photographed under a phase-contrast microscope at 12, 24, and 48 h. Experiments were performed in triplicate.

Transwell chambers (Corning, Corning, NY, USA) were used to evaluate cell migration and invasion. For the migration assay, transfected cells (2.5×10^4 cells/well) were seeded into a Transwell plate (Corning, Corning, NY, USA). For the invasion assay, 2.5×10^4 transfected cells were plated in each well of a Corning BioCoat Matrigel Invasion Chamber (Corning, NY). In the upper insert, cells were suspended in a serum-free medium with 10 % FBS medium in the lower chamber as a chemoattractant. After 18–24 h of incubation, migrated or invaded cells were fixed and stained. Different fields of view were photographed, and cells were counted. All experiments were performed in triplicates.

2.7. RNA preparation and quantitative real-time PCR

Total RNA was extracted using a TRIzol reagent. cDNA synthesis was performed using a PrimeScript RT reagent kit (Takara, Otsu, Japan). mRNA expression level of each gene was quantified using Power SYBR Green (TaKaRa) on ABI Prism 7900HT (Applied Biosystems, Foster City, CA, USA) and then calculated with the $2^{-\Delta\Delta Ct}$ method. *GAPDH* was used as the internal control for normalization. Analyses were performed in triplicates for each gene. The primers used in this study are listed in Supplementary Table 1.

2.8. Western blotting

Cell lysates were prepared by incubating cell pellets in lysis buffer (50 mmol/l Tris–HCl, pH 8.0; 150 mmol/l NaCl, 0.5 % NP-40) supplemented with protease inhibitors and phosphatase inhibitors, followed by centrifugation at 14,000×*g* for 15 min at 4 °C. Equal amounts of proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes. The membranes were incubated with primary antibodies at 4 °C overnight, followed by incubation with secondary antibodies at room temperature for 1 h. Immunoreactive bands were detected using a western blotting luminol reagent (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). The primary antibodies include SOX8 (1:500, Cat.A3131, Abclonal), Cyclin D1(1:1000, Cat.A19038, Abclonal), Vimentin (1:500, Cat.A19607, Abclonal), C-myc (1:500, Cat.A19032, Abclonal), β-catanin (1:1000, Cat.A19657, Abclonal), TWIST1 (1:500, Cat.A15596, Abclonal), GSK3β (1:500, Cat.A11731, Ablonal), *p*-GSK3β-S9 (1:500, Cat.AP1088, Abclonal), FZD6 (1:500, Cat.A10503, Abclonal), E-Cadherin (1:500, Cat.A19038, Abclonal), OCT4 (1:500, Cat.ab19857, Abcam), ABCG2 (1:1000, Cat.A17908, Abclonal), and GAPDH (1:2000, Cat.1610374, Bio-rad).

2.9. Indirect immunofluorescence

Indirect immunofluorescence analysis of HCT116 and SW620 cells was performed as previously described [26,27]. HCT116 and SW620 cells transfected with shSOX8 or shControl were seeded on coverslips. After 24 h, cells were fixed with 4 % formaldehyde and incubated with primary antibody against E-Cadherin and Vimentin at 4 °C overnight, and then stained with fluorescein isothiocyanate-conjugated secondary antibody against mouse (Cat.F0313; Dako, Glostrup, Denmark). Cell nuclei were stained with 4, 6-diamidino-2-phenylindole and imaged using a fluorescence microscope (Leica, Germany).

2.10. Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed using an Active Motif ChIP-IT Express Kit (Cat.53008; Carlsbad, CA). For each ChIP reaction, total chromatin (20 μg) was incubated with Protein G magnetic beads (25 μL; Invitrogen) and SOX8 primary antibody (1 μg; 1:200, Cat.374446, Santa Cruz Biotechnology) overnight at 4 °C. A QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) was used to purify the precipitated DNA, and real-time PCR was performed to detect the enrichment of targeted DNA segments. Primers used for the ChIP assay are listed in Supplementary Table 2.

2.11. TCF/LEF reporter assay

To measure the transcriptional activity of the β-catenin luciferase reporter, TOPFlash vector (Biovector, Beijing, China) and pRL Renilla luciferase control reporter vector were co-transfected into HCT116 and SW620 cells, respectively. After 48 h of transfection, a Dual-Luciferase Reporter Assay Kit (Promega, Madison, Wisconsin, USA) was used to detect luciferase activity.

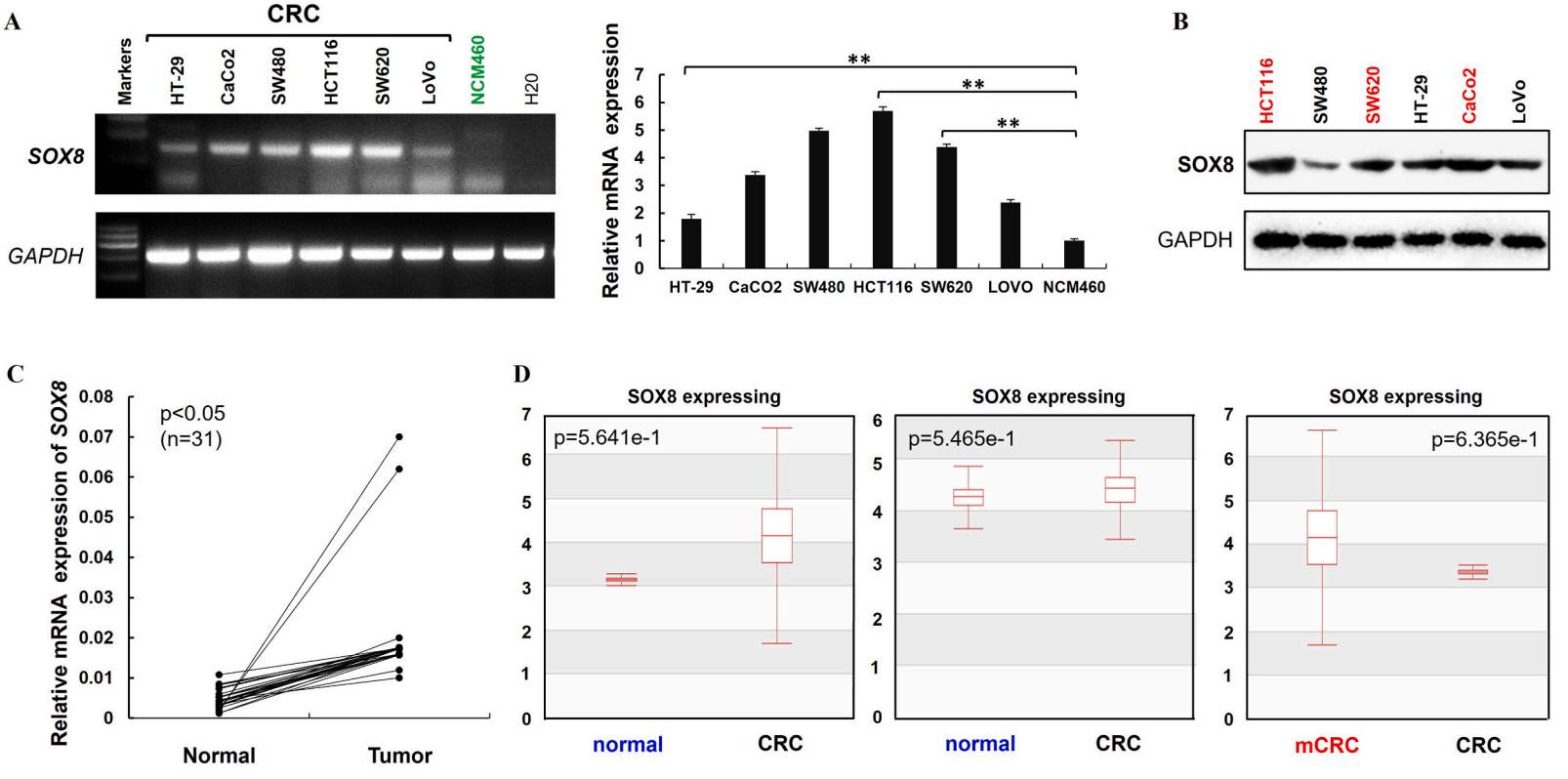

Fig. 1. SOX8 expression in colorectal cancer cell lines and tissue samples. (A) mRNA expression levels of *SOX8* in colorectal cancer cell lines and human normal colon cells detected by RT-PCR (left) and qRT-PCR (right), with *GAPDH* as internal control. (B) SOX8 protein level in CRC cell lines. (C) Relative *SOX8* expression level in CRC patient samples and normal controls. (D) *SOX8* expression presented by HCMDB database. mCRC: metastatic colorectal cancer.

2.12. Xenograft mouse model

Animal experiments were carried out following the Animal Research Committee guidelines of Peking University-Shenzhen Hospital. Cultured cells (1×10^7 cells/ml) were used to generate mouse xenografts. HCT116 cells with shSOX8 and shControl were suspended in DMEM (50 μL) with 10 % FBS and implanted subcutaneously into the flank of three 6-week-old female nude mice (GemPharmatech Company, Guangdong, China). After injection, tumor volumes were evaluated every 4 days. Mice were sacrificed on day 28; tumors were collected and weighed. Tumor volumes (mm^3) were calculated as tumor volume = 0.5 × (longest diameter) ×

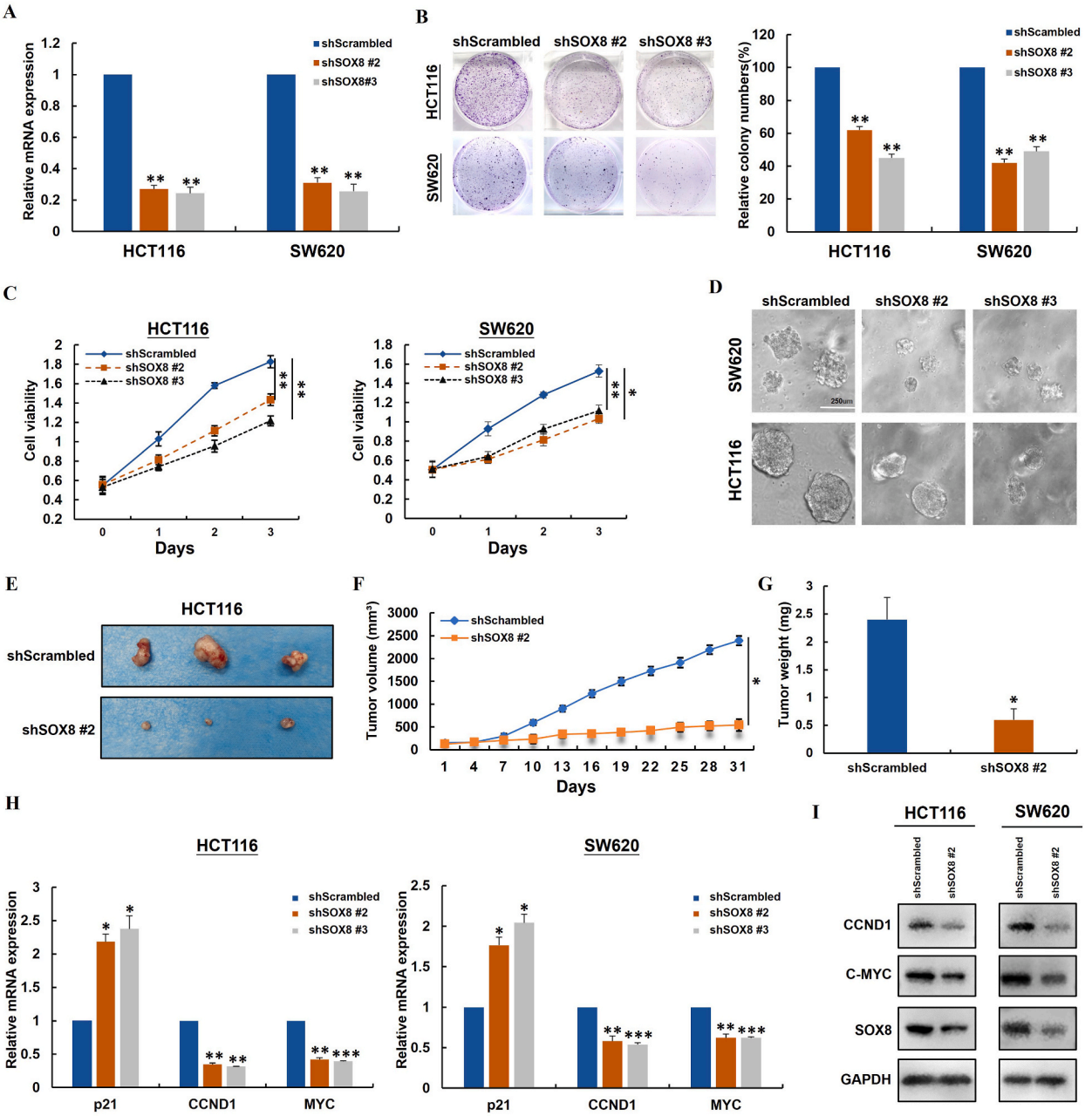

Fig. 2. Decreased expression of SOX8 accompanied with decreased CRC cell growth by possibly regulating cell cycle. (A) Knockdown of SOX8 in HCT116 and SW620 cell lines by sh-RNAs was confirmed at the mRNA level by qPCR. (B) Cell proliferation ability was tested by colony formation assay. (C) Representative CCK8 assay in HCT116 and SW620 cells with SOX8 knockdown. (D) Representative pictures of 3D culture in HCT116 and SW620 cells. The representative pictures (E) and the growth curves of tumors (F) derived from HCT116 and SW620 cells. (G) The final weight of tumors derived from HCT116 and SW620 cells with SOX8 knockdown compared with control cells. (H) Detection of the mRNA expression of cell cycle regulators *p21, CCND1* and *C-MYC* by real-time PCR. (I) Detection of the protein expression of CCND1 and C-MYC by Western blot.

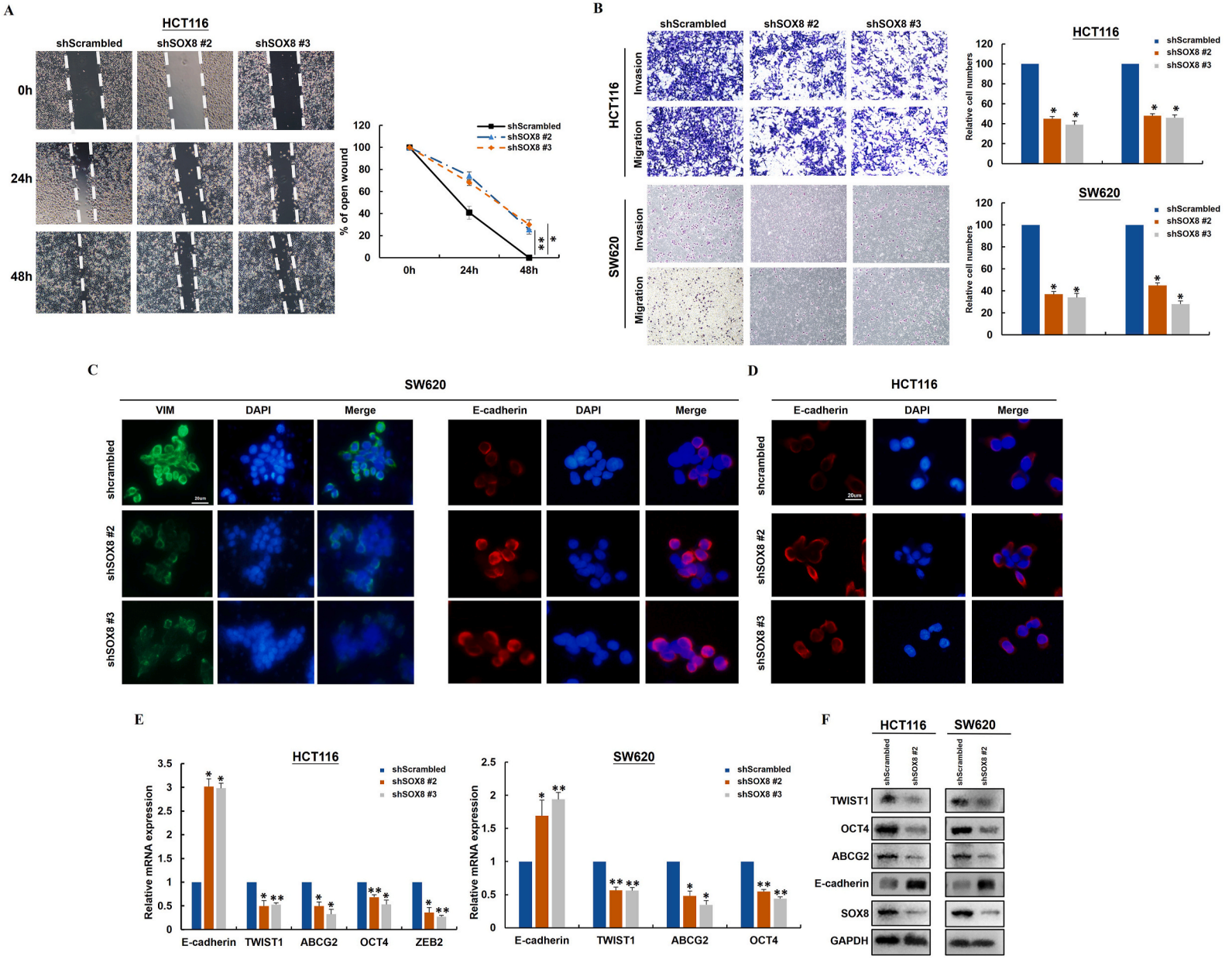

Fig. 3. Knockdown of SOX8 accompanied with reduced ability of migration and invasion in CRC cells. (A)Wound healing assay showed the wound closure rate of HCT116 and SW620 cells with SOX8 knockdown compared with controls. (B) Transwell migration and invasion assay of HCT116 and SW620 cells with SOX8 knockdown and negative controls. Migrated cells at the lower surface of the transwell filter were stained (left panel) and counted (right panel). Immunofluorescence staining of vimentin and E-cadherin in shSOX8 SW620 (C) and HCT116 (D) cells and negative controls. (E) mRNA expression of several EMT markers and stem cell markers assessed by qRT-PCR in shSOX8 SW620 and HCT116 cells compared to negative controls. (F) Exploration of the protein expression levels of several EMT markers and stem cell markers by Western blot in shSOX8 SW620 and HCT116 cells compared to negative controls.

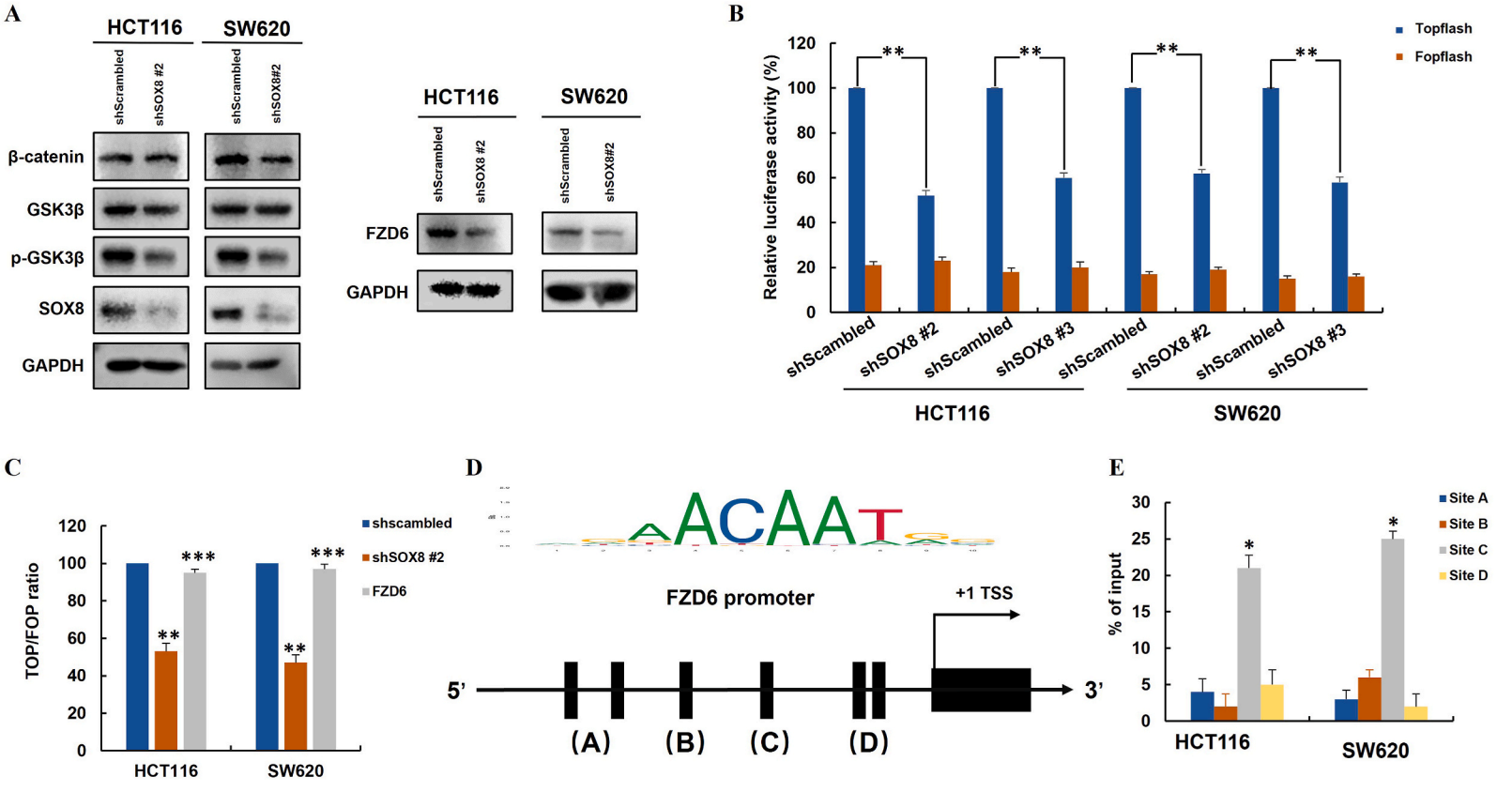

Fig. 4. SOX8 regulated Wnt/β-catenin signaling in FZD6 dependent manner. (A) Left panel: Exploration the protein expression of Wnt/β-catenin signaling markers. *GAPDH* was used as loading control. Right panel: Western blot analyzed the expression of FZD6 protein in shSOX8 and shscrambled HCT116 and SW620 cells. (B) Analysis of the activity of Wnt/β-catenin signaling by dual-luciferase reporter assay in shSOX8 and shscrambled HCT116 and SW620 cells. (C) Analysis of the activity of Wnt/β-catenin signaling by dual-luciferase reporter assay in shscrambled, shSOX8 with FZD6 re-expression or not HCT116 and SW620 cells. (D) Predicted transcriptional binding sequence of SOX8 by JASPAR program (upper). Schematic diagram of predicted SOX8 binding sites in the FZD6 promoter. (E) A chromatin immunoprecipitation (ChIP) assay was performed in HCT116 and SW620 cells using an anti-SOX8 antibody or IgG as negative control.

(shortest diameter)^2.

2.13. Statistical analysis

Results were presented as mean ± SD unless otherwise indicated. Statistical comparisons were analyzed with Student's t-test. Statistical significance was set at $P < 0.05$. Significance. For all comparisons, the criteria for significance were $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

3. Results

3.1. Upregulation of SOX8 in CRC cell lines and clinical samples

Firstly, we evaluated *SOX8* expression in CRC cell lines. Reverse transcription (RT)-PCR analysis showed that *SOX8* was remarkably upregulated in multiple CRC cell lines (Fig. 1A, left), which was consistent with the results of real-time quantitative PCR (Fig. 1A, right). In accordance with PCR results, we found higher protein expression of SOX8 in HCT116 and SW620 cells (Fig. 1B). In clinical samples, higher mRNA expression of *SOX8* was detected in tumor samples than in adjacent normal tissues (Fig. 1C). Using the online HCMDB database (http://hcmdb.i-sanger/index), we found than CRC tumor samples exhibited higher SOX8 expression than normal colon tissues. In addition, metastatic CRC tissues showed higher SOX8 expression than primary tumor tissues (Fig. 1D). These data suggest that *SOX8* overexpression is a frequent event in CRC, indicating its oncogenic role in CRC development.

3.2. SOX8 promotes tumor cell proliferation possibly by regulating the cell cycle

To investigate the role of *SOX8* in tumorigenesis, we assessed its effect on cell proliferation. We generated three shRNAs targeting *SOX8*, which effectively downregulated *SOX8* expression (Fig. 2A and Supplementary Fig. 1). The colony formation assay showed that loss of *SOX8* expression decreased the number of colonies in HCT116 and SW620 cells (Fig. 2B). CCK8 assays indicated that HCT116 and SW620 cells with *SOX8* knockdown had a lower proliferation rate than control cells (Fig. 2C). In the 3D culture system, HCT116 and SW620 with *SOX8* knockdown showed decreased anchorage-independent growth (Fig. 2D). To confirm these effects, we generated xenograft tumors in nude mice. We observed that reduced *SOX8* expression significantly inhibited xenograft growth compared to the control (Fig. 2E and F). Xenograft tumor weight was also significantly lower in the shSOX8 group than in the control group (Fig. 2G). To assess the mechanism by which *SOX8* knockdown inhibits tumor cell growth, we examined whether *SOX8* knockdown affected the expression of cell cycle regulators. Real-time PCR analysis showed that *SOX8* knockdown in HCT116 and SW620 cells significantly increased the expression of *p21* and decreased the expression of *CCND1* and *C-MYC* (Fig. 2H). Western blotting confirmed that CCND1 and C-MYC were downregulated at the protein level in HCT116 and SW620 cells with *SOX8* knockdown (Fig. 2I). Collectively, these data indicate that SOX8 affects the expression of cell cycle regulators and promotes tumor cell growth, possibly by inducing cell cycle arrest.

3.3. SOX8 knockdown reverses EMT and stemness in CRC cells

Next, we explored the role of *SOX8* in tumor metastasis. The wound healing assay showed that *SOX8* knockdown significantly suppressed the wound closure rate in HCT116 and SW620 cells (Fig. 3A). Transwell migration and invasion assays indicated that *SOX8* inhibited HCT116 and SW620 migration and invasion ability (Fig. 3B). We evaluated the expression of multiple key EMT markers as EMT plays a critical role in tumor cell metastasis. Immunofluorescence assays revealed that knockdown of *SOX8* increased E-cadherin levels (Fig. 3C and D) but suppressed vimentin expression (Fig. 3C). Real-time PCR and western blotting confirmed that *SOX8* knockdown decreased E-cadherin expression at both mRNA and protein levels (Fig. 3E and F). SOX8 knockdown also inhibited *ZEB2* expression, a negative regulator of E-cadherin. Furthermore, real-time PCR and western blotting showed that reduced *SOX8* expression downregulated multiple stem cell markers, including *ABCG2*, *TWIST1,* and *OCT4* (Fig. 3E and F). These results demonstrate that SOX8 can prevent tumor metastasis, possibly by impairing the EMT program and inhibiting stemness.

3.4. SOX8 regulated WNT/β-catenin signaling on FZD-6 dependent manner

Wnt/β-catenin signaling plays a critical role in the development of CRC, with more than 90 % of CRC cases harboring alterations in Wnt components [28–30]. SOX8 is one of the crucial regulators for Wnt/β-catenin signaling. Here, we observed a reduced level of phospho-GSK3β in both HCT116 and SW620 cells with shSOX8 compared with their corresponding parental cells (Fig. 4A left panel). In addition, the knockdown of *SOX8* in HCT116 and SW620 decreased the activities of Wnt/β-catenin signaling shown by β-catenin reporter assay (Fig. 4B). We explored the connection between SOX8 and Wnt/β-catenin signaling. The FZD family is a typical receptor of Wnt signaling, and SOX8 binds to the promoter region of FZD7 in TSCC cells and regulates FZD7 expression. We investigated whether SOX8 affects the expression of *FZD7* in CRC. We screened the transcriptional expression of FZD members in HCT116 cells with *SOX8* knockdown. However, we found that *FZD6* but not *FZD7* mRNA levels were markedly reduced (Supplementary Fig. 2). Immunoblotting confirmed that SOX8 knockdown downregulated FZD6 expression (Fig. 4A, right panel). Next, we overexpressed *FZD6* in HCT116 and SW620 cells with *SOX8* knockdown. Using a dual-luciferase reporter assay, we found that the re-expression of *FZD6* partially recovered the Wnt/β-catenin signaling activity (Fig. 4C). Overexpression of *FZD6* in SOX8 knockdown stable SW620

cells induced increased expression of phospho-GSK3β (Supplementary Fig. 3A). In addition, we confirmed the decreased expression of FZD6 in transplanted tumor samples from SOX8 knockdown stable HCT116 cells (Supplementary Fig. 3B). Ectopic expression of *FZD6* significantly increased the colony formation ability of SW620 cells with SOX8 downregulation (Supplementary Fig. 3C). These data strongly suggest that FZD6 plays a critical role in SOX8-mediated WNT/β-catenin signaling. According to the potential binding site provided by the JASPAR program (https://jaspar.genereg.net/), we found several possible binding sites of SOX8 in the upstream region of the FZD6 promoter (Fig. 4D) [31]. Using chromatin immunoprecipitation (ChIP) analysis, we confirmed the binding of SOX8 to cluster C of FZD6 (Fig. 4E). In summary, our data proved that SOX8 regulated the Wnt/β-catenin signaling activity in an FZD6-dependent manner.

4. Discussion

Morbidity and mortality rates of CRC are increasing worldwide. Most patients are diagnosed at advanced stage and miss the best treatment time. Therefore, screening for potential biomarkers for the early detection of CRC is urgently needed. Members of the SOX family act as oncogenes or tumor suppressors during cancer progression. A recent study showed that *SOX8* expression was associated with the T stage and could be an independent prognostic factor for patients with CRC [32]. In this study, we found that SOX8 was highly expressed in CRC cell lines and tumor samples, which is consistent with the findings retrieved from the HCMDB database. Knockdown of *SOX8* inhibits cell proliferation, migration, and invasion, suggesting the oncogenic roles of SOX6 in CRC development. Mechanistically, SOX8 inhibited CRC cell proliferation by modulating the expression of several cell cycle regulators such as *CCND1* and *C-MYC*. We also found decreased expression of oncogenes and stem cell markers, including *TWIST1*, *ABCG2,* and *ZEB2*, implying that EMT and stemness may be involved in the regulation of CRC metastasis. Based on the potential binding sequence of SOX8 provided by the JASPAR program, we found *CCND1*, *C-MYC*, *TWIST1*, *ABCG2*, and *ZEB2* had several binding sites in their promoter regions, suggesting that SOX8 might directly regulate their expression as a transcription factor. In conclusion, we identified knockdown of *SOX8* inhibited growth, metastasis, EMT phenotype, and stemness through regulating the expression of several crucial genes that might be direct SOX8 targets in CRC.

The Wnt/β-catenin signaling pathway was closely related to the occurrence, progression, and prognosis of CRC [33]. FZDs are transmembrane proteins that interact with Wnt ligands [21]. FZD6 has been shown to regulate both canonical and non-canonical Wnt pathways, although it tends to be more prevalent in the non-canonical Wnt pathways. As a key regulator of the Wnt pathway, FZD6 is highly expressed in multiple types of human cancers, including breast and colorectal cancer [34–37]. Overexpression of *FZD6* in breast cancer increases cell invasion and metastasis, which is associated with a worse prognosis in patients [34,38]. In glioblastoma and neuroblastoma, high *FZD6* expression results in tumor growth in mouse xenografts and is associated with a worse prognosis [39]. However, the precise role of FZD6 in CRC has not been elucidated. In the present study, we found that SOX8 binds to the promoter region of *FZD6* and regulates FZD6 expression. The expression of SOX8 and FZD6 is highly correlated in vivo and vitro study in CRC cells. Ectopic expression of FZD6 partly reversed the growth inhibitory effect in SW620 cells with SOX8 downregulation. These results provide evidence of a new role of FZD6 in CRC, which has important implications for the development of potential target-based therapies for patients with CRC. Therefore, more functional and mechanical studies including overexpression of SOX8 or FZD6 deserve further exploration and validation in the future work in CRC.

In summary, our study demonstrated that SOX8 serves as the key regulator of CRC cell growth, and metastasis by activating FZD6-dependent Wnt/β-catenin signaling. These findings suggest that SOX8 is a promising therapeutic target for CRC.

5. Data availability statement

Due to confidential issues, the datasets generated and/or analyzed during the current work are not publicly available but are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Chen Li: Writing – original draft. **Boran Cheng:** Writing – review & editing. **Xiaodong Yang:** Methodology. **Gangling Tong:** Validation. **Fen Wang:** Data curation. **Mengqing Li:** Data curation. **Xiangyu Wang:** Validation. **Shubin Wang:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We thank Mr.Tang Mengxiong and the staff of the central animal facility for technical assistance; Prof. Wang Shubin was supported by Shenzhen Science and Technology Innovation Commission Project (ZDSYS20190902092855097, KCXFZ202002011010508, and GJHZ20200731095207023). Dr. Cheng Boran was supported by National Natural Science Foundation of China (81602489), Basic Research Program of Shenzhen Innovation Council (JCYJ20210324105609024). This study was also supported by Medical Scientific Research Foundation of Guangdong Province of China (A2023274).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22586.

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