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Original Research Article

ANKRD22 enhances cancer stem cell growth and cisplatin resistance in cervical cancer via NUSAP1/Wnt/β-catenin pathway

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Abstract

Purpose: *To investigate the role of ankyrin repeat domain 22 (ANKRD22) and nucleolar spindle-associated protein 1 (NUSAP1)-mediated Wnt/β-catenin pathway in cervical cancer (CC).*
Methods: *ANKRD22 levels were evaluated in tissue samples collected from CC patients. The CC cells were transfected with an ANKRD22 silencing vector. Cell proliferation and migration were determined via colony formation, BrdU, scratch and Transwell assays. Sphere formation test was performed to determine the effect of ANKRD22 on the cancer stem cell (CSC)-like traits of CC cells. The effect of ANKRD22 on CSC-like traits was further evaluated by determining the expressions of the CSC markers, Oct4, SOX2, and Nanog, while cisplatin resistance was assessed by CCK-8 assay. The effect of ANKRD22 on the NUSAP1-mediated Wnt/β-catenin pathway was evaluated by determining the expression levels of β-catenin, matrix metalloproteinase-7, and adenomatous polyposis coli. Moreover, ANKRD22-mediated tumor growth was monitored in vivo using an animal model.*
Results: *ANKRD22 was overexpressed in cancer tissues from CC patients ($p < 0.05$). ANKRD22 knockdown suppressed CC cell proliferation, migration, invasion, and CSC traits, and also positively regulated Wnt/β-catenin pathway through NUSAP1 ($p < 0.05$). However, the inhibition of ANKRD22 suppressed tumor growth in vivo through NUSAP1. In addition, the silencing of ANKRD22 alleviated cisplatin resistance in CC cells ($p < 0.05$).*
Conclusion: *ANKRD22 activates NUSAP1/Wnt/β-catenin pathway, and enhances CSC-like characteristics and cisplatin resistance, thus exacerbating the malignant behaviors of CC cells. Therefore, ANKRD22 could serve as a promising target for CC treatment.*

Keywords: *Cervical cancer, Cancer stem cells, Ankyrin repeat domain 22, Nucleolar spindle-associated protein 1/Wnt/β-catenin pathway, Cell migration and invasion*

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INTRODUCTION

Cervical cancer (CC) is a common gynecological cancer, and the estimated age-standardized worldwide incidence was 13.3 % in 2020 [1]. Cancer stem cells (CSC) are enriched inside the

population cells and can self-renew and differentiate [2,3]. The study on CSC in CC has been expanded to identify candidate biomarkers. In particular, cisplatin resistance is one of the main limitations of clinical efficacy [2].

Ankyrin repeat domain 22 (ANKRD22) is expressed specifically in normal digestive tract epithelium and tumor cells. Yin *et al.* identified ANKRD22 as a new tumor-related gene involved in non-small cell lung cancer (NSCLC) [4]. Pan *et al*. found that ANKRD22 is upregulated in colorectal cancer-initiating cells, and ANKRD22 transports excess lipids to mitochondria, and reduces the number of mitochondria in an autophagy-independent manner [5]. In addition, ANKRD22 may be involved in prostate cancer [6]. ANKRD22 stimulates cell growth in breast cancer by regulating nucleolar spindle-associated protein 1 (NUSAP1) and activating the Wnt/β-catenin pathway [7]. It has also been shown that ANKRD22 is upregulated in endometrial cancer [8]. However, the role of ANKRD22 in the progression of CC remains unclear.

NUSAP1 is a microtubule-binding protein that regulates mitosis, and spindle formation and stability. NUSAP1 has been reported to be associated with CC [9], which was upregulated in cervical adenocarcinoma, and enhanced CSC characteristics through Wnt/β-catenin signaling, thereby promoting CC cell metastasis [10]. The Wnt/β-catenin pathway mediates CC progression [11], and high β-catenin expression is a poor prognostic factor for CC [12]. Although it has been shown that NUSAP1/Wnt/β-catenin pathway is involved in CC, it is unclear whether ANKRD22 is involved in the regulation of NUSAP1/Wnt/β-catenin pathway, thus affecting CC progression.

This study aimed to investigate the molecular mechanisms underlying the role of ANKRD22 in CC, and also to determine the relationship between ANKRD22 and NUSAP1/Wnt/β-catenin pathway in CC and cisplatin resistance.

METHODS

Collection of clinical samples and ethical approval

Cervical cancer tissues and adjacent normal tissues were obtained from 50 patients, who were diagnosed with CC upon case examination at Taizhou Municipal Hospital (Taizhou, Jiangsu Province, China). This study complied with the guidelines of the Declaration of Helsinki [13]. Written informed consents were obtained from patients, and the study was approved by the Ethics Committee of Taizhou Municipal Hospital (approval no. 2021KTWST024). Patients with other tumors, receiving radiotherapy and chemotherapy for CC, and have acute inflammation of the reproductive tract were excluded from the study. Tissue samples were washed in sterile saline, frozen in liquid nitrogen for 15 min, and then stored in a refrigerator at -80 °C.

Cell culture

Cervical cancer cell lines HeLa (CRM-CCL-2), Ca Ski (CRM-CRL-1550), SiHa (HTB-35), C-33A (HTB-31), and normal human cervical epithelial cells End1/E6E7 (CRL-2615), were obtained from ATCC (VA, USA). End1/E6E7 cells were cultured in keratinocyte serum-free medium (Gibco, CA, USA) containing 0.05 mg/mL bovine pituitary extract, 0.1 ng/mL human recombinant epidermal growth factor (EGF), and 44.1 mg/L calcium chloride [14]. HeLa, SiHa, and C-33A cells were cultured in Eagle's Minimum Essential Medium (Gibco). Ca-Ski cells were cultured in Roswell Park Memorial Institute 1640 medium (Thermo Fisher Scientific, MA, USA). Each medium was supplemented with 10 % fetal bovine serum (FBS), 100 mg/mL streptomycin and 100 units/mL penicillin (Gibco).

Cell transfection

The vectors targeting ANKRD22 (si-ANKRD22#1 and si-ANKRD22#2) and the corresponding negative control (si-NC) were designed by GenePharma (Shanghai, China). Vector (600 ng) was transfected into HeLa and Ca Ski cells with Lipofectamine 3000 (Thermo Fisher Scientific) for 24 h [15].

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

After isolation of RNA using a TRIzol kit (Invitrogen, CA, USA), reverse transcription was performed using the PrimeScript RT Reagent Kit (Takara, Dalian, China). RT-PCR was performed using SYBR Premix Ex Taq™ II (Takara) and the Bio-Rad CFX-96 detection system (Bio-Rad, CA, USA). ANKRD22 expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression and determined by the $2^{-\Delta\Delta Ct}$ method. The primers used were obtained from Sangon (Shanghai, China).

Western blot assay

Tissues and cells were lysed with radio-immunoprecipitation assay lysis buffer, protein

concentrations of lysates were quantified using a bicinchoninic acid protein kit, and proteins (20 µg) were separated using 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes.

The membranes were incubated with the following primary antibodies (Abcam, Cambridge, MA, USA): anti-ANKRD22 (1: 1000, ab165151), anti-Oct4 (1: 1000, ab181557), anti-SOX2 (1: 1000, ab92494), anti-Nanog (1: 1000, ab109250), anti-NUSAP1 (1 µg/mL, ab169083), anti-β-catenin (1: 10000, ab32572), anti-APC (adenomatous polyposis coli, 1: 5000, ab40778), anti-MMP-7 (matrix metalloproteinase-7, 1: 1000, ab207299), and anti-GAPDH (1: 1000, ab8245) overnight at 4 °C. Then, the membrane was incubated with a secondary antibody conjugated with horseradish peroxidase (HRP; 1:1000, CST, MA, USA) and detected.

Colony formation assay

HeLa and Ca Ski cells (5×10^3 cells/well in 6-well plates) were cultured in Eagle's Minimum Essential Medium and RPMI1640 medium for two weeks. Thereafter, the colonies were fixed with 4 % paraformaldehyde, stained with 0.5 % crystal violet solution for 2 h, and counted.

5-Bromo-2'-dexoyuridine (BrdU) test

HeLa and Ca Ski cells were treated with BrdU solution (BD Pharmingen, CA, USA) and cultured for 4 h. After fixing with methanol and blocking with rabbit serum, cells were probed with anti-BrdU (Abcam) for 2 h, followed by staining with 4',6-diamidino-2-phenylindole (BD Pharmingen, San Diego, CA, USA) and microscopic imaging under a fluorescence microscope (Olympus, Tokyo, Japan).

Scratch test

HeLa and Ca Ski cells (1×10^6 cells/mL) at 80–90 % confluence were scratched evenly using a 10 µL sterile pipette tip and cultured further in complete medium. Cell images were taken at 0 and 24 h.

Transwell assay

HeLa and Ca Ski cells were mixed with serum-free medium, and cell suspensions (1×10^5 cells/mL, 200 µL) were placed in the upper chambers of the Transwell® chambers (Corning, Beijing, China) coated with Matrigel (BD Biosciences, NJ, USA). After 24 h, cells were attracted to the 10 % FBS in the lower chamber were fixed with 4 % paraformaldehyde, stained with 0.1 % crystal violet solution, and counted under a microscope.

Formation of tumor spheroids

Cancer stem cells (CSCs) of HeLa and Ca Ski cells were sorted [16] and cultured in Dulbecco's modified Eagle's medium/F12 medium containing N2 and B27 (Invitrogen), 20 ng/mL basic fibroblast growth factor, and 20 ng/mL EGF for 1–2 weeks. During this period, medium was changed every two days. The formed spheroids were detached with 0.25 % trypsin-ethylene diamine tetra-acetic acid and viewed under a microscope.

Determination of cell viability

HeLa and SiHa cells were cultured with increasing concentrations of cisplatin (Sigma, St. Louis, MO, USA) for more than 6 months to establish cisplatin-resistant cell lines, and the resistant cell lines were HeLa/DDP and SiHa/DDP. Cell viability was evaluated with a CCK-8 kit (Bimake, Houston, USA). Cells were seeded in 96-well plates at ~10^4 cells/well and incubated with CCK-8 solution for 1.5 h. Absorbance was measured at 450 nm with a microplate reader.

Tumor xenografts from nude mice

BALB/C of nude mice (4 weeks old, 18 – 22 g) were purchased from Wenzhou Medical University (Wenzhou, Zhejiang Province, China). HeLa cells were suspended in a 1:1 mixture of PBS and Matrigel (1×10^6 cells/200 µL) and cells were then injected into the back of the right hind leg of a nude mouse that had been anesthetized with pentobarbital sodium. Tumor volumes were monitored once a week. After 5 weeks, tumors were excised and weighed. This animal study was conducted in accordance with the "Guidelines for the Care and Use of Laboratory Animals" [17] and was approved by the Ethics Committee of Taizhou Municipal Hospital (approval no. 2021KTWST024).

Immunohistochemistry (IHC)

Tumor sections were deparaffinized and rehydrated with xylene in the ethanol gradient. Sections were incubated with rabbit anti-ANKRD22 (1:1000, 031890-ML405, US Biological, MA, USA), anti-NUSAP1 (1:1000, ab247043, Abcam), and anti-Ki-67 (1 µg/mL, ab15580, Abcam) overnight. Then, the sections were probed with HRP-conjugated goat anti-

rabbit immunoglobulin G (1:1000, CST) and examined under a microscope (Olympus).

Statistical analysis

Data analysis was performed using SPSS 22.0 (IBM, NY, USA). Measurement data were presented as mean ± standard deviation. The *t*-test was used for comparison between the two groups, while one-way analysis of variance (ANOVA) was used for comparisons among multiple sets of data. Tukey's multiple comparisons test was conducted for pairwise comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

ANKRD22 was overexpressed in CC

The potential role of ANKRD22 in CC was evaluated using the Gene Expression Profiling Interactive Analysis (GEPIA) website (http://gepia.cancer-pku.cn/detail.php), and the results showed that ANKRD22 was highly expressed in CC (Figure 1 A). To verify the results from bioinformatics analysis, qRT-PCR and Western blot were performed to determine ANKRD22 expression in clinical tissues from 50 CC patients, and the results showed that ANKRD22 mRNA and protein expressions was higher in CC tissues than in adjacent normal tissues ($P<0.01$, Figure 1 B and C). In addition, ANKRD22 mRNA and protein expressions were higher in the CC cell lines HeLa, Ca Ski, SiHa, and C-33A than in normal human cervical epithelial End1/E6E7 cells ($p < 0.01$; Figure 1 D and E). The highest ANKRD22 expression was found in HeLa and Ca Ski cell lines, which were chosen for further experiments. Taken together, ANKRD22 was overexpressed in CC tissues and cells.

Knockdown of ANKRD22 blocked the proliferation, migration, and invasion of CC cells

To determine the biological functions of ANKRD22 in CC, ANKRD22 was knocked down in HeLa and Ca Ski cells by transfection with si-ANKRD22#1 or si-ANKRD22#2 (Figure 2 A). The colony formation and BrdU assays showed the decreased proliferative rate of HeLa and Ca Ski cells upon the silencing of ANKRD22 (Figure 2 B and C). Transwell and scratch assays showed decreased invasion and migration of HeLa and Ca Ski cells after the knockdown of ANKRD22 ($P<0.001$, Figure 2 D and E). Taken together, ANKRD22 knockdown inhibited the proliferation, migration, and invasion of CC cells.

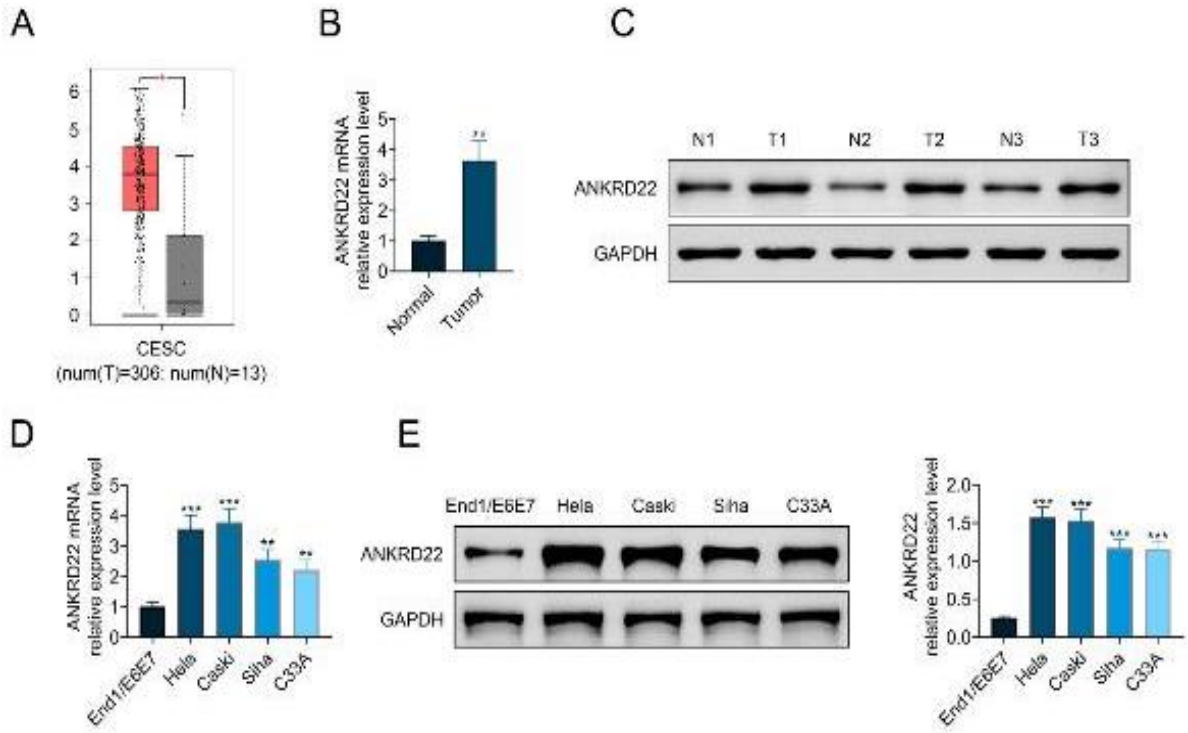

Figure 1: ANKRD22 was overexpressed in CC. A) ANKRD22 expression in CC predicted by GEPIA. B, C) ANKRD22 expression in CC tissues and normal tissues as measured by RT-qPCR and Western blot, n = 50. D, E) ANKRD22 expression in HeLa, Ca Ski, SiHa, and C-33A CC cells and in normal human cervical epithelial End1/E6E7 cells, as measured by RT-qPCR and Western blot. Experiments were repeated three times. ** $P < 0.01$, *** $p < 0.001$ vs. normal tissues or End1/E6E7 cells

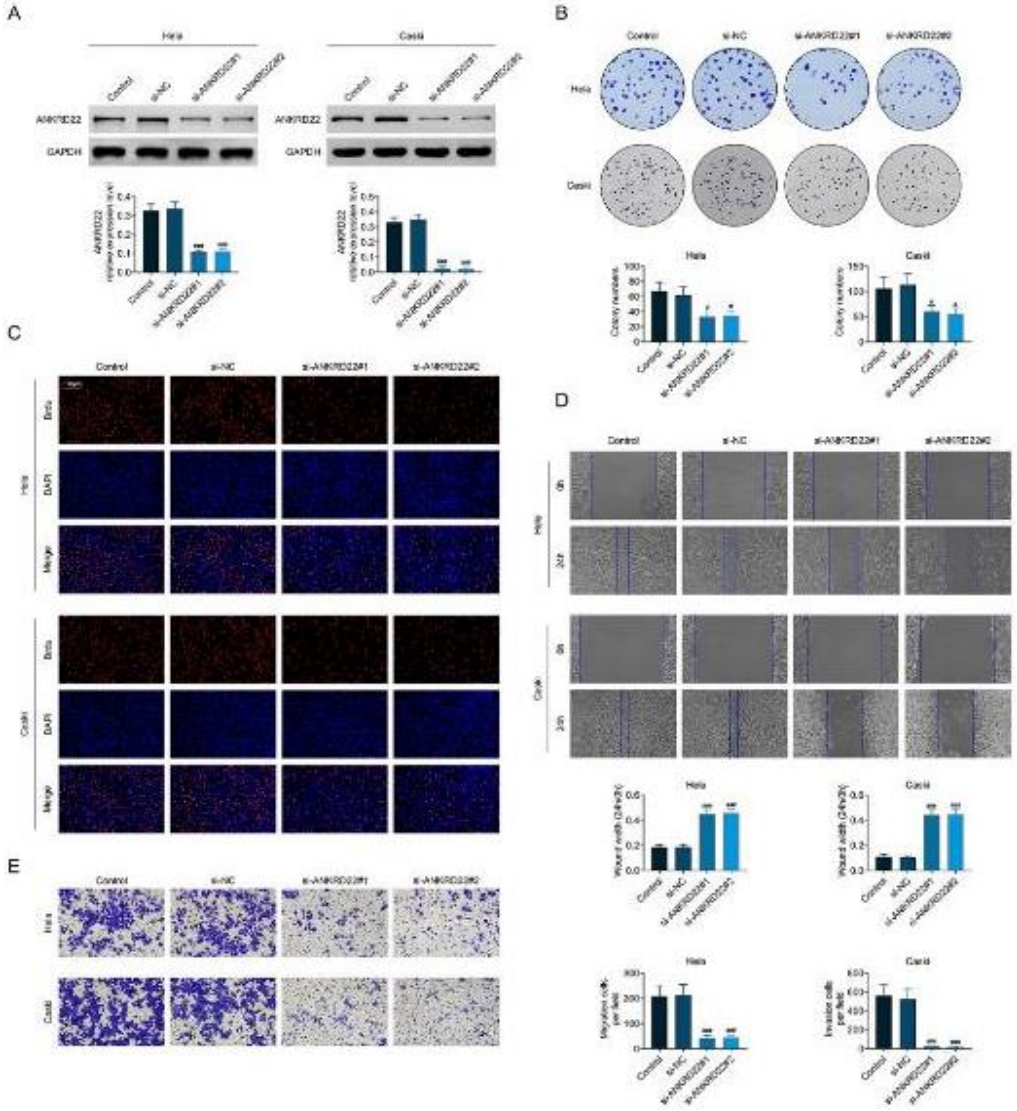

Figure 2: Loss of ANKRD22 blocks proliferation, migration, and invasion of CC cells. A) ANKRD22 expression in HeLa and Ca Ski cells after transfection with si-ANKRD22 as evaluated by Western blot. B) The number of colonies formed was determined by the colony formation test. C) Cell proliferation as assessed with the BrdU test. D. Cell migration was evaluated by the scratch test. E) Cell invasion was evaluated by the transwell assay. Experiments were repeated three times. * $P < 0.05$, ***$p < 0.001$ vs. si-NC group

ANKRD22 silently inhibited the CSC-like characteristics of CC cells

The tumor spheroid formation test and Western blot results showed that the spheroid formation ability of CSCs was decreased (Figure 3 A) and the protein levels of CSC markers Oct4, SOX2, and Nanog were decreased after ANKRD22 knockdown (P<0.01, Figure 3 B). Taken together, ANKRD22 knockdown inhibited the CSC-like properties of CC cells.

ANKRD22 silencing alleviated cisplatin resistance in CC cells

To determine the role of ANKRD22 in cisplatin resistance in CC cells, HeLa and SiHa cells were incubated with different concentrations (2.5, 5.0, 10, 20, 40, 80, 160, 320 and 640 μg/mL) of cisplatin to obtain cisplatin-resistant cells. The cisplatin IC_{50} of HeLa and SiHa cells was respectively lower than that in cisplatin-resistant cells (Figure 4 A). The knockdown of ANKRD22 decreased the cisplatin IC_{50} in cisplatin-resistant HeLa and SiHa cells (P<0.001, Figure 4 B). Thus, ANKRD22 knockdown significantly relieved cisplatin resistance in CC cells.

ANKRD22 activated NUSAP1-dependent Wnt/β-catenin pathway

To uncover the mechanism underlying the effects of ANKRD22 knockdown on CC cells, NUSAP1 levels in HeLa and Ca Ski cells were measured

by Western blot, and it was observed that the downregulation of ANKRD22 reduced NUSAP1 protein expression (*P*<0.01, Figure 5 A and B). To determine whether ANKRD22 regulates the Wnt/β-catenin pathway through NUSAP1, the protein expressions in the Wnt/β-catenin pathway was investigated. The knockdown of ANKRD22 reduced the protein expressions of β-catenin and MMP-7, but induced the protein expression of the tumor suppressor APC (Figure 5 A and B). These results suggested that ANKRD22 positively regulated Wnt/β-catenin pathway through NUSAP1.

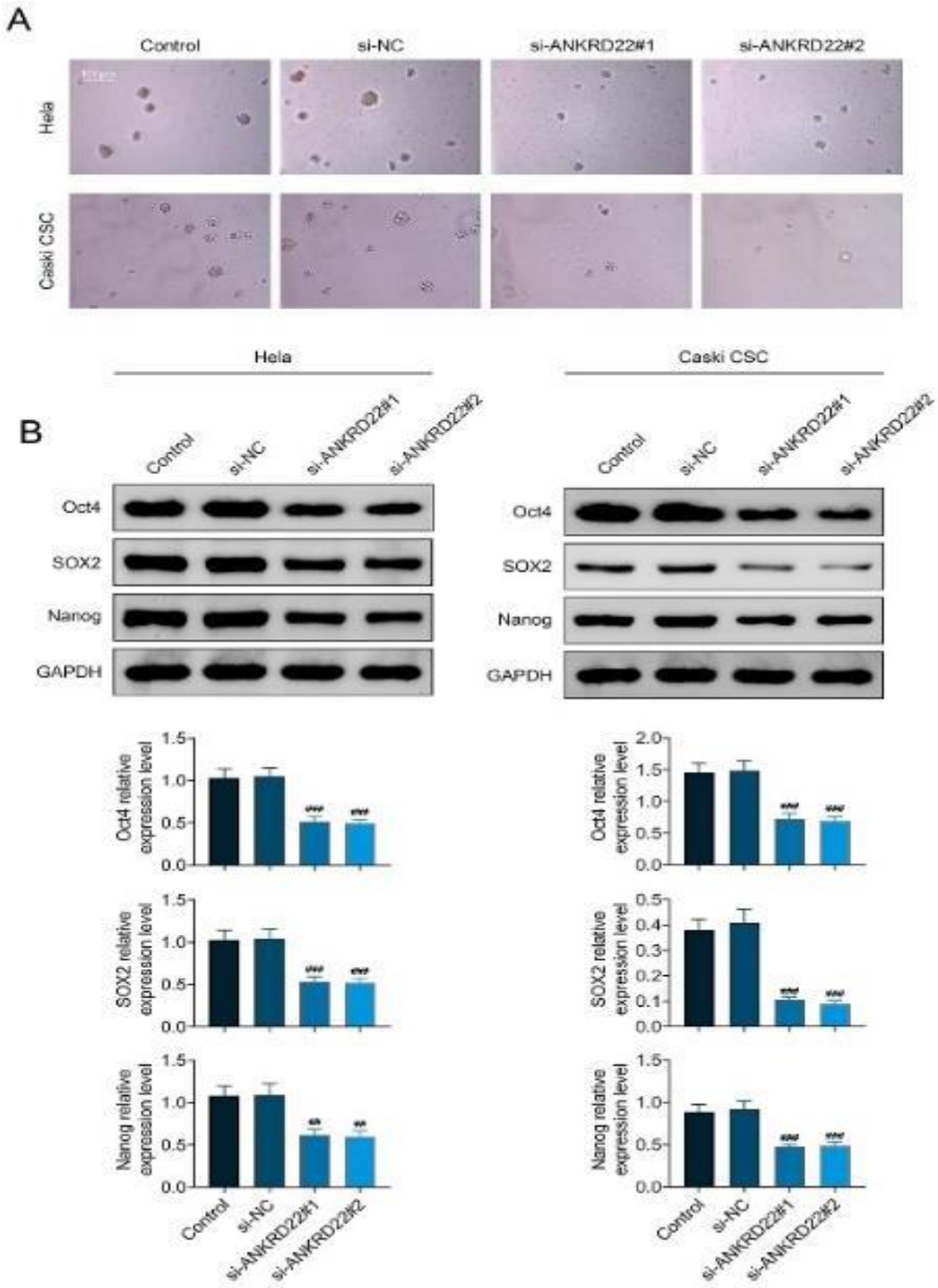

Figure 3: ANKRD22 silencing inhibited CSC-like characteristics. A) Cell self-renewal ability was detected as the spheroid formation test. B) Oct4, SOX2, and Nanog protein levels in Hela and Ca Ski cells were measured by Western blot. Experiments were repeated three times. * *P* < 0.05, *** *p* < 0.001 vs. the si-NC group

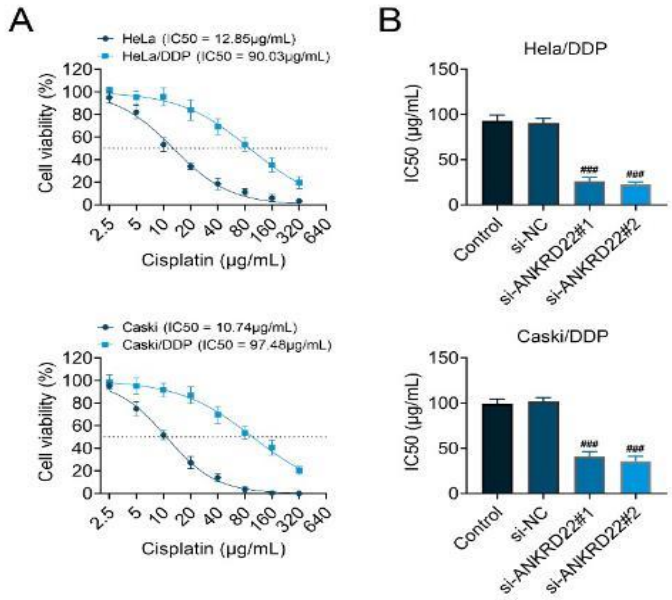

Figure 4: ANKRD22 silencing alleviated cisplatin resistance in CC cells. A) Cell viability in response to cisplatin was determined by CCK-8 assay. B) Cell viability in response to ANKRD22 silencing and cisplatin was detected by CCK-8 assay. Experiments were repeated three times. #*P* < 0.05, ###*p* < 0.001 vs. si-NC and cisplatin groups

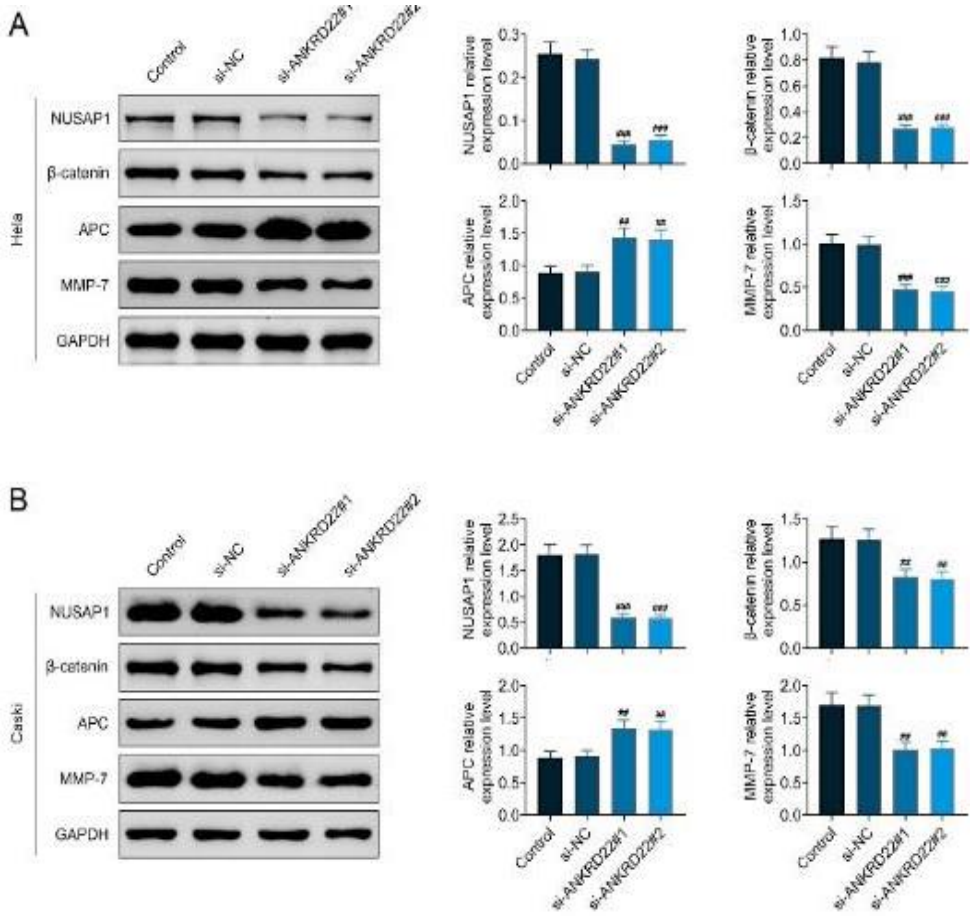

Figure 5: ANKRD22 activates NUSAP1/Wnt/β-catenin pathway. A, B) Protein levels of NUSAP1 and Wnt/β-catenin pathway-related proteins β-catenin, APC, and MMP-7 as measured by Western blot. Experiments were repeated three times. ***P* < 0.01, ****p* < 0.001 vs. si-NC group

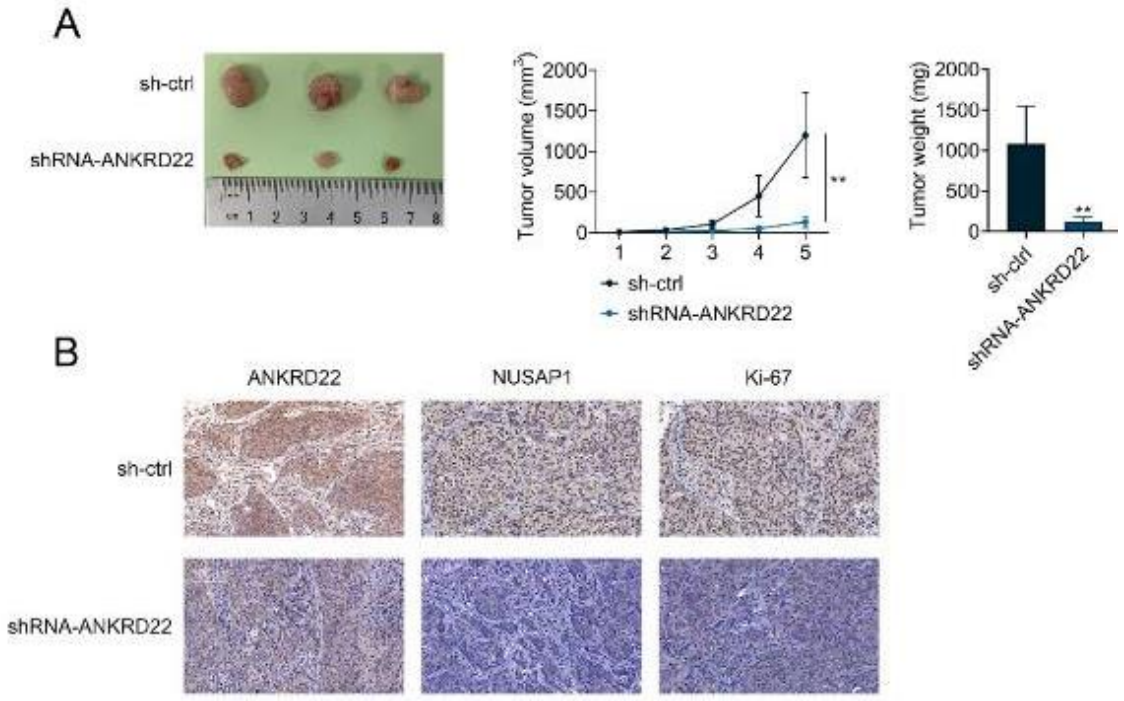

Figure 6: Downregulation of ANKRD22 suppressed CC cell growth *in vivo*. A) Representative images of tumors in nude mice. B) Statistical analysis of the tumor volume in nude mice. C) Statistical analysis of the tumor weight in nude mice. D) ANKRD22, NUSAP1, and Ki-67 levels in tumors were evaluated IHC. n = 5, $^{**}p < 0.01$ vs. sh-ctrl group

ANKRD22 knockdown suppressed the growth of CC cells *in vivo*

A mouse xenograft tumor model was established to determine whether ANKRD22 acts as a tumor promoter *in vivo*. The depletion of ANKRD22 decreased the tumor volume and tumor weight in mice ($P<0.01$, Figure 6 A). IHC analysis of tumors revealed that the downregulation of ANKRD22 decreased the protein expressions of ANKRD22, NUSAP1, and Ki-67 *in vivo*. (Figure 6 D). Therefore, the downregulation of ANKRD22 suppressed tumor growth *in vivo*.

DISCUSSION

Approximately two-thirds of patients with CC are diagnosed at an advanced stage, and usually have low survival rate. To explore the molecular mechanism of CC, the role of ANKRD22, an oncogene in various cancers [4,7], was investigated in this study. The results showed that ANKRD22 promotes the development of CC by regulating the NUSAP1/Wnt/β-catenin pathway and increasing the CSC-like characteristics of cervical cells. Cisplatin resistance is gradually emerging and is now one of the main limitations of clinical efficacy and CC progression in some patients [18,19]. ANKRD22 plays a key role in the cisplatin resistance of CC cells.

This study showed that ANKRD22 is highly expressed in clinical cancer tissues and CC cell lines. A previous study showed the role of ANKRD22 in colorectal cancer-initiating cells. Increased levels of ANKRD22 have been found in prostate cancer tissues [6], which is consistent with our results. In this study, ANKRD22 was knocked down in CC and ANKRD22 silencing inhibited the proliferation, invasion, migration, and CSC-like characteristics of CC cells *in vitro*. ANKRD22 silencing also alleviated cisplatin resistance in CC cells. Moreover, the downregulation of ANKRD22 decreased tumor volume and weight, as well as the expressions of NUSAP1 and Ki-67 in CC tumors, thus confirming the tumor promoter role of ANKRD22 in CC *in vivo*. Similarly, the downregulation of ANKRD22 inhibited breast cancer cell proliferation and invasion [7]. In NSCLC, the suppression of ANKRD22 blocked cell proliferation [4]. However, the precise role of ANKRD22 in mediating the CSC-like characteristics of tumor cells remains to be assessed.

Next, the malignant effects of ANKRD22 on CC cells were investigated. ANKRD22 silencing downregulated NUSAP1 expression, thereby blocking the activation of the Wnt/β-catenin pathway. ANKRD22 has been shown to switch Wnt/β-catenin pathway on and off through NUSAP1 [7]. The knockdown of NUSAP1

inhibited CC development by obstructing the Wnt/β-catenin pathway. A previous study demonstrated NUSAP1-dependent tumor progression [20]. In addition, NUSAP1 is a negative indicator of patients' prognosis, and the tumor-promoting effects of NUSAP1 could be inhibited by downregulating NUSAP1 in gastric cancer. Furthermore, a previous study demonstrated that NUSAP1 overexpression resulted in the restoration of ladder cancer cell proliferation, migration, and invasion that were inhibited by miR-769-5p [9]. The downregulation of NUSAP1 also inhibited the growth and metastasis of NSCLC cells.

The Wnt/β-catenin pathway is hyperactivated in CC, and activation of Wnt/β-catenin pathway leads to CC cell tumorigenicity [21]. The silencing of NUSAP1 in triple-negative breast cancer cells limits cell proliferation and invasion by inhibiting Wnt/β-catenin pathway. In addition, down-regulation of NUSAP1 inhibited nasopharynx-geal carcinoma cell proliferation and invasion by blocking Wnt/β-catenin pathway [10]. NUSAP1 is an inducer of CSC traits in CC cells [10], and NUSAP1 knockdown inhibited the CSC traits of cancer cells and blocked Wnt/β-catenin pathway [10]. During NSCLC cell development, NUSAP1, an oncogene, positively activated Wnt/β-catenin pathway, thereby facilitating tumor progression [18]

CONCLUSION

This study demonstrates that ANKRD22 enhances CSC characteristics and cisplatin resistance of CC cells through NUSAP1/Wnt/β-catenin axis. This finding provides a promising basis for the clinical development of targeted agents for the treatment of CC.

DECLARATIONS

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Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dan Pan designed the study and supervised the data collection; Wenwei Ye analyzed and interpreted the data; Xiaoxiao Qiu and Yaxian Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

REFERENCES

1. *Wahid M, Dar SA, Jawed A, Mandal RK, Akhter N, Khan S, Khan F, Jogiah S, Rai AK, Rattan R. Microbes in gynecologic cancers: Causes or consequences and therapeutic potential. Semin Cancer Biol 2021.*
2. *Batlle E, Clevers H. Cancer stem cells revisited. Nat Med 2017; 23(10): 1124-1134.*
3. *Hao MH, Zhang F, Liu XX, Zhang F, Wang LJ, Xu SJ, Zhang JH, Ji HL, Xu P. Qualitative and quantitative analysis of catechin and quercetin in flavonoids extracted from Rosa roxburghii Tratt. Trop J Pharm Res 2018; 17(1): 71-76.*
4. *Yin J, Fu W, Dai L, Jiang Z, Liao H, Chen W, Pan L, Zhao J. ANKRD22 promotes progression of non-small cell lung cancer through transcriptional up-regulation of E2F1. Sci Rep 2017; 7(1): 4430.*
5. *Pan T, Liu J, Xu S, Yu Q, Wang H, Sun H, Wu J, Zhu Y, Zhou J, Zhu Y. ANKRD22, a novel tumor microenvironment-induced mitochondrial protein*

promotes metabolic reprogramming of colorectal cancer cells. Theranostics 2020; 10(2): 516-536.

6. *Qiu Y, Yang S, Pan T, Yu L, Liu J, Zhu Y, Wang H. ANKRD22 is involved in the progression of prostate cancer. Oncol Lett 2019; 18(4): 4106-4113.*
7. *Wu Y, Liu H, Gong Y, Zhang B, Chen W. ANKRD22 enhances breast cancer cell malignancy by activating the Wnt/beta-catenin pathway via modulating NuSAP1 expression. Bosn J Basic Med Sci 2021; 21(3): 294-304.*
8. *Wang A, Guo H, Long Z. Integrative Analysis of Differently Expressed Genes Reveals a 17-Gene Prognosis Signature for Endometrial Carcinoma. Biomed Res Int 2021; 2021(4804694.*
9. *Chen Y, Zhang W, Kadier A, Zhang H, Yao X. MicroRNA-769-5p suppresses cell growth and migration via targeting NUSAP1 in bladder cancer. J Clin Lab Anal 2020; 34(5): e23193.*
10. *Li H, Zhang W, Yan M, Qiu J, Chen J, Sun X, Chen X, Song L, Zhang Y. Nucleolar and spindle associated protein 1 promotes metastasis of cervical carcinoma cells by activating Wnt/beta-catenin signaling. J Exp Clin Cancer Res 2019; 38(1): 33.*
11. *Ke L, Chen Y, Li Y, Chen Z, He Y, Liu J, Zhuang Y. miR-142-5p promotes cervical cancer progression by targeting LMX1A through Wnt/beta-catenin pathway. Open Med (Wars) 2021; 16(1): 224-236.*
12. *Zhuang S, Yu X, Lu M, Li Y, Ding N, Ding Y. High mobility group box 3 promotes cervical cancer proliferation by regulating Wnt/beta-catenin pathway. J Gynecol Oncol 2020; 31(6): e91.*
13. *Association WM. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization 2001; 79(4): 373.*
14. *Zhang J, He H, Wang K, Xie Y, Yang Z, Qie M, Liao Z, Zheng Z. miR-326 inhibits the cell proliferation and cancer stem cell-like property of cervical cancer in vitro and oncogenesis in vivo via targeting TCF4. Ann Transl Med 2020; 8(24): 1638.*
15. *Yao Z, Shu L, Yi Y, Qiao L. Hsa_circRNA_000543 Predicts Poor Prognosis and Promotes Cervical Cancer Cell Progression Through Regulating miR-567/ZNF268 Axis. Cancer Manag Res 2021; 13(5211-5222.*
16. *Zhang L, Guo C, Ji T, Chen X. SOX2 Regulates lncRNA CCAT1/MicroRNA-185-3p/FOXP3 Axis to Affect the Proliferation and Self-Renewal of Cervical Cancer Stem Cells. Nanoscale Res Lett 2021; 16(1): 2.*
17. *Council NR. Guide for the care and use of laboratory animals. 2010.*
18. *Xie Q, Ou-Yang W, Zhang M, Wang H, Yue Q. Decreased Expression of NUSAP1 Predicts Poor Overall Survival in Cervical Cancer. J Cancer 2020; 11(10): 2852-2863.*
19. *Marquina G, Manzano A, Casado A. Targeted Agents in Cervical Cancer: Beyond Bevacizumab. Curr Oncol Rep 2018; 20(5): 40.*
20. *Zhao Y, He J, Li Y, Lv S, Cui H. NUSAP1 potentiates chemoresistance in glioblastoma through its SAP domain to stabilize ATR. Signal Transduct Target Ther 2020; 5(1): 44.*
21. *Chen M, Li L, Zheng PS. SALL4 promotes the tumorigenicity of cervical cancer cells through activation of the Wnt/beta-catenin pathway via CTNNB1. Cancer Sci 2019; 110(9): 2794-2805.*