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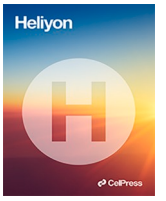

Research article

SIAH2 is specifically expressed during cervical carcinogenesis, and closely relates to the abnormal proliferation of cervical epithelial cells

Li-ping Jing [b,1], Meng Li [a,1], Xi-yan Xia [c], Xin Zheng [a], Jia-yu Chen [a], Jing He [d], Xue-wei Zhuang [a,*]

[a] Department of Laboratory, Shandong Provincial Third Hospital, Shandong University, 250031, Jinan, Shandong, China
[b] Clinical Laboratory Department, Liaoning Cancer Hospital & Institute, 110042, Shenyang, Liaoning, China
[c] Department of Immunology Teaching and Research, Jinan Vocational College of Nursing, 250102, Jinan, Shandong, China
[d] Second Clinical Medical College, Shandong University of Traditional Chinese Medicine, 250014, Jinan, Shandong, China

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ABSTRACT

Background: Cervical cancer is one of the most common malignancies in women worldwide. As a RING type ubiquitin ligase, SIAH2 has been reported to promote the progression of a variety of tumors by interacting with and targeting multiple chaperones and substrates. The aim of this study was to further identify the role and the related molecular mechanisms involved of SIAH2 in cervical carcinogenesis.

Methods and results: Cellular assays *in vitro* showed that knockdown of SIAH2 inhibited the proliferation, migration and invasion of human cervical cancer cells C33A and SiHa, induced apoptosis, and increased the sensitivity to cisplatin treatment. Knockdown of SIAH2 also inhibited the epithelial-mesenchymal transition and activation of the Akt/mTOR signaling pathway in cervical cancer cells, which were detected by Western blot. Mechanistically, SIAH2, as a ubiquitin ligase, induced the ubiquitination degradation of GSK3β degradation by using coIP. The results of complementation experiments further demonstrated that GSK3β overexpression rescued the increase of cell proliferation and invasion caused by SIAH2 overexpression. Specific expression of SIAH2 appeared in precancerous and cervical cancer tissues compared to inflammatory cervical lesions tissues using immunohistochemical staining. The more SIAH2 was expressed as the degree of cancer progressed. SIAH2 was significantly highly expressed in cervical cancer tissues (44/55, 80 %) compared with precancerous tissues (18/69, 26.1 %). Moreover, the expression level of SIAH2 in cervical cancer tissues was significantly correlated with the degree of cancer differentiation, and cervical cancer tissues with higher SIAH2 expression levels were less differentiated.
Conclusion: Targeting SIAH2 may be beneficial to the treatment of cervical cancer.

* Corresponding author. Department of Laboratory, Shandong Provincial Third Hospital, Shandong University, No.11, Wuyingshan Middle Road, Tianqiao District, 250031, Jinan, Shandong, China.
E-mail address: zhuangxuewei@sdu.edu.cn (X.-w. Zhuang).
[1] Both authors have contributed equally.

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1. Introduction

In 2018, the World Health Organization called for global action (HPV vaccination and cellular screening) to eliminate cervical cancer by the end of the 21st century. However, cervical cancer remains the fourth most common and deadly-causing cancer in women [1]. According to global estimates for 2020, there were 604,000 new cases and 342,000 deaths of cervical cancer [1]. Currently, surgery and radiotherapy remain the mainstay of treatment for non-metastatic cervical cancer, and the addition of chemotherapy to radiotherapy may improve patient survival [2]. Studies on the molecular biology of cervical cancer have revealed several potential therapeutic targets, and several new drugs developed in this context, including targeted therapies (anti-VEGF and anti-EGFR) and immunotherapies (pabolizumab and navulizumab), are being evaluated, which may be beneficial for prolonging patient survival, especially for patients with recurrent and metastatic cervical cancer [2]. Continued exploration of key proto-oncogenes and therapeutic targets in cervical cancer is useful and necessary.

Vertebrate SIAH2 belongs to of the highly evolutionarily conserved RING type E3 ligases family, and is expressed in numerous human epithelial cell lines, interacting with and targeting a wide range of substrates and chaperones to orchestrate ubiquitin-mediated protein hydrolysis [3–5]. Recent studies revealed that SIAH2 functions as a pro-oncogenic factor in prostate, breast, colon, and gastric cancers, and could regulate cancer cell proliferation, growth, apoptosis, stemness, chemo-sensitivity and resistance, adhesion and polarity, invasion, and migration in both HIF-1α-dependent and non-dependent manners [6–10]. In addition, SIAH2 inhibitor and metabolic antagonist have been shown to delay prostate cancer progression [11]. However, the role of SIAH2 in cervical cancer remains unclear.

In this research, we will analyze the specific expression and role of SIAH2 in the progression of human cervical cancer based on clinical cervical tissue samples and *in vitro* culture of human cervical cancer cell lines, in an attempt to provide a new target for gene therapy of cervical cancer.

2. Materials and methods

2.1. Cell culture and transfection

Human cervical cancer cell lines C33A and SiHa were purchased from the Cell Bank of the Chinese Academy of Sciences. Cells were cultured in 1640 Medium (Gibco-BRL, USA) supplemented with fetal bovine serum (10 %) (Thermo Fisher Scientific, China) at 37 °C with 5 % CO_2. SIAH2 specific siRNA was designed and synthesized from GeneChem Co., Ltd (Shanghai, China). Lipofectamine 2000 (Invitrogen, USA) was used for the transfection. Briefly, 2.5 μg of SIAH2 siRNA and 5 μg of Lipofectamine 2000 reagent were diluted in 200 μL of serum- and antibiotic-free medium, respectively. After standing at room temperature for 15 min, the two dilutions were mixed and stood for another 20 min. Subsequently, the mixture was added dropwise to cells growing at 60 % confluence. Six hours later, old medium was removed and new medium was added, after which normal culture and further experimental assays could be performed.

2.2. Cell counting Kit-8 (CCK8) assay

Cell proliferation and survival were determined by the CCK8 assay. For cell proliferation detection, transfected cells were seeded into 96-well plates at a concentration of 1×10^4 cells. Cell viability was measured every 24 h using the CCK8 reagent purchased from Solarbio Science & Technology. After the incubation with 10-μL CCK8 reagent at 37 °C for 1.5 h, the optical density was detected at 450 nm. For cell survival detection under cisplatin (DDP) treatment, 1×10^8 transfected cells were inoculated into a 96-well plate. Different concentrations of cisplatin were added to the wells, and the assay was performed after 24 h of incubation. Cisplatin (HY-17394) was purchased from MedChemExpress.

2.3. Colony formation assay

Transfected cells were seeded into six-well plates at a density of 500 cells/well. The plates were cultured for 14 days. Then, cells were fixed with 4 % paraformaldehyde for 15 min, and stained with gentian violet. The generated colonies were photographed and counted.

2.4. Transwell assay

Transwell chambers (Thermo Fisher Scientific, China) were placed in a 24-well plate with 1640 medium supplemented with 10 % FBS prepared at the bottom. 5000 cells were transferred to the upper chamber with serum-free 1640 medium. After 24 h of incubation, migrated or invasive cells were fixed with 4 % paraformaldehyde for 15 min, and stained with crystal violet for 30 min. The images were captured at 100× magnification. For the invasion assay, pre-colded Matrigel was uniformly spread in the transwell chambers.

2.5. Wound healing assays

After cell transfection, SiHa and C33A cells were seeded on a six-well plate and scratched with a 200 μl micropipette tip. After 24 h and 48 h of incubation in serum-free medium, cells were washed with PBS buffer. The images were captured at 40× magnification.

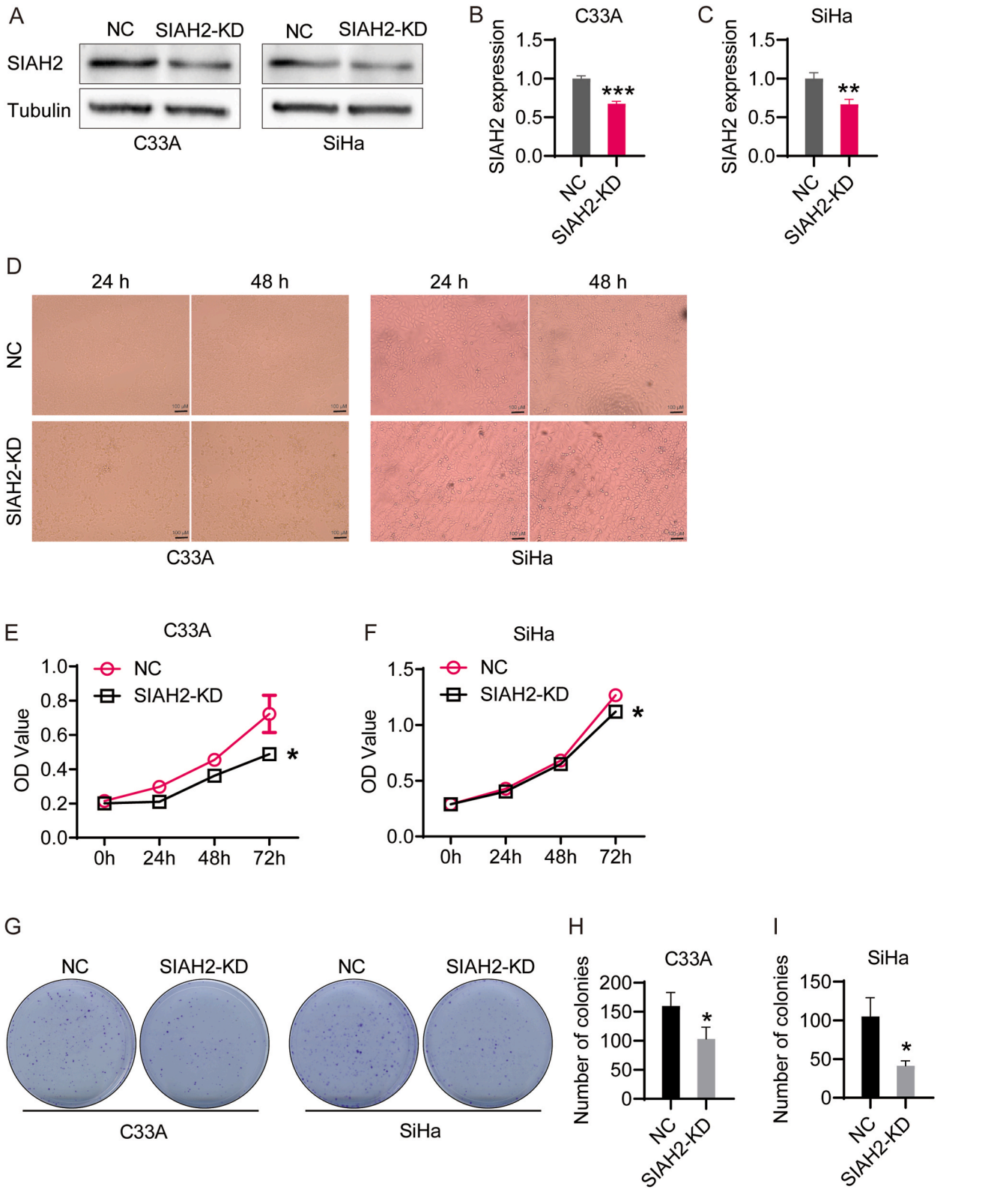

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Fig. 1. SIAH2 knockdown inhibits the proliferation of cervical cancer cells.
(A) SIAH2 siRNA was transfected into C33A and SiHa cells. The original images of Western blot are listed in Supplementary Material 1. Western blot was performed to detected the expression of SIAH2 in C33A (B) and SiHa (C) cells. (D) Microscopic cell morphology images of 24 and 48 h after SIAH2 siRNA transfection. The proliferation of C33A (E) and SiHa (F) cells was detected using CCK8 assay. (G) Colony formation assay was performed to further confirm the anti-proliferation effect of SIAH2 knockdown. Number of colonies of C33A (H) and SiHa (I) were counted after 14 days of incubation. *P < 0.05, **P < 0.01, ***P < 0.001.

Wound healing area was analyzed by ImageJ software.

2.6. Western blot and immunoprecipitation

Proteins were obtained using a RIPA buffer (Solarbio Science & Technology, China) containing protease inhibitor and phosphatase inhibitor after 24 h of transfection. Then, proteins were separated by SDS-PAGE, and transferred to a polyvinylidene difluoride membrane. Then, the membrane was blocked with fat-free milk, and subjected to immunoprobing with specific antibodies. An enhanced chemiluminescence kit (Thermo Fisher Scientific, USA) was used for the visualization of protein bands. Antibodies against SIAH2 (12651-1-AP), GSK3β (22104-1-AP), Caspase9 (10380-1-AP), Caspase3 (66470-2-Ig), Bax (50599-2-Ig), Bcl-2 (26593-1-AP), *p*-mTOR (67778-1-Ig), mTOR (66888-1-Ig), Vimentin (10366-1-AP), N-cadherin (22018-1-AP), E-cadherin (20874-1-AP), Snail1 (13099-1-AP) and Tubulin (11224-1-AP) were purchased from Proteintech Group, Inc. (Chicago, USA). Antibodies against *p*-AKT (ab38449), AKT (ab8805) were purchased from Abcam (Cambridge, UK). The relative protein levels were analyzed using ImageJ software.

Immunoprecipitation was performed using anti-Myc (Sigma, USA) or HA (Sigma, USA) antibody-coupled agarose beads for 3 h at 4 °C. The immunoblotting was consistent with Western blot method.

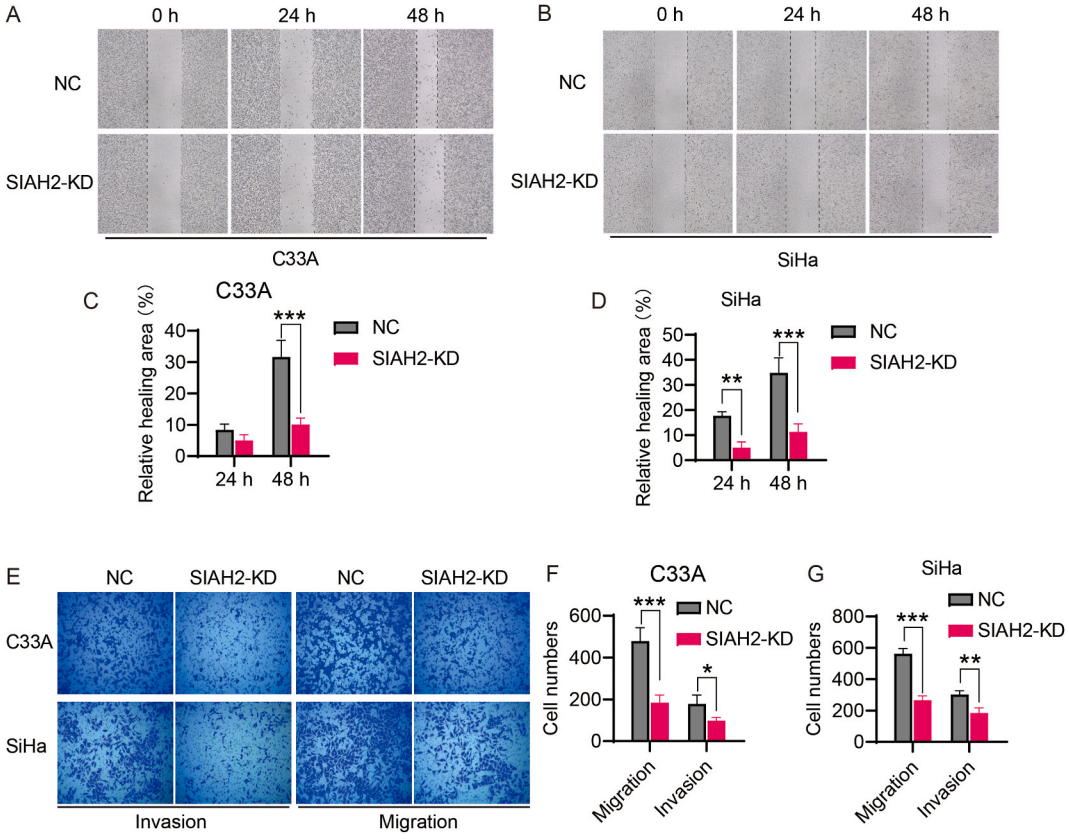

Fig. 2. SIAH2 knockdown inhibits the migration and invasion of cervical cancer cells.
(A and B) Wound healing assay was performed to detect the migration of C33A and SiHa cells. The relative healing area of C33A (C) and SiHa (D) cells was analyzed after 48 h of scratch. (E) Transwell assay was performed to further confirm the effect of SIAH2 on cell migration and invasion. The migrated or invasive cell number of C33A (F) and SiHa (G) cells was counted in 3 random fields. *P < 0.05, **P < 0.01, ***P < 0.001.

2.7. Tissues and immunohistochemical (IHC) stain

The cervical tissues of 6 cervicitis, 69 cervical intraepithelial neoplasia, and 55 cervical carcinoma patients were obtained from Shandong University Affiliated Shandong Provincial Third Hospital in 2018.

Cervical tissues were fixed overnight in neutral formaldehyde solution, subsequently embedded in paraffin and cut into 4 μm thick sections. After microwave antigen repair, sections were incubated with anti-SIAH2 antibody for 2 h at room temperature, followed by incubation with secondary antibody solution for 30 min at room temperature. Color reactions were performed using DAB solution. Staining was scored on a scale of A (degree of cell staining) × B (percentage of colored cells). A: 0 (no staining), 1 (light yellow), 2 (brownish yellow), 3 (dark brown). B: 1 (1–10 %), 2 (11–50 %), 3 (51–80 %), 4 (>80 %). A score of 0 is negative, 1–4 is weakly positive, 5–7 is strongly positive, and ≥8 is highly expressed.

2.8. Statistical analysis

All data in this research was generated from three independent experiments and presented as mean ± SD. SPSS 18.0 software was used for the statistical analysis. The student t-test and one-way ANOVA was used for the comparison between groups. P value < 0.05 was considered statistically significant.

3. Results

3.1. SIAH2 knockdown inhibits the proliferation of cervical cancer cells

To elucidate the role of SIAH2 in cervical cancer, we knocked down SIAH2 in C33A and SiHa cells using siRNA interference. The protein level of SIAH2 was significantly inhibited after the transfection of siRNA-SIAH2 according to the results of Western blot both in C33A and SiHa cells (Fig. 1A–C). The morphology of the cells transfected for 24 and 48 h was slightly wrinkled (Fig. 1D). CCK8 assay proved that silencing SIAH2 significantly inhibited the proliferation in C33A (Fig. 1E) and SiHa (Fig. 1F) cells. Colony formation assay was performed to further confirm the anti-proliferation effect of SIAH2 knockdown (Fig. 1G). Compared with the control group, SIAH2 knockdown reduced the number of colonies both in C33A (Fig. 1H) and SiHa (Fig. 1I) cells. The results implied that SIAH2 knockdown suppressed the proliferation of cervical cancer cells.

3.2. SIAH2 knockdown inhibits the migration and invasion of cervical cancer cells

Wound healing assay was performed to detect the migration of C33A (Fig. 2A) and SiHa (Fig. 2B) cells. Our results uncovered that

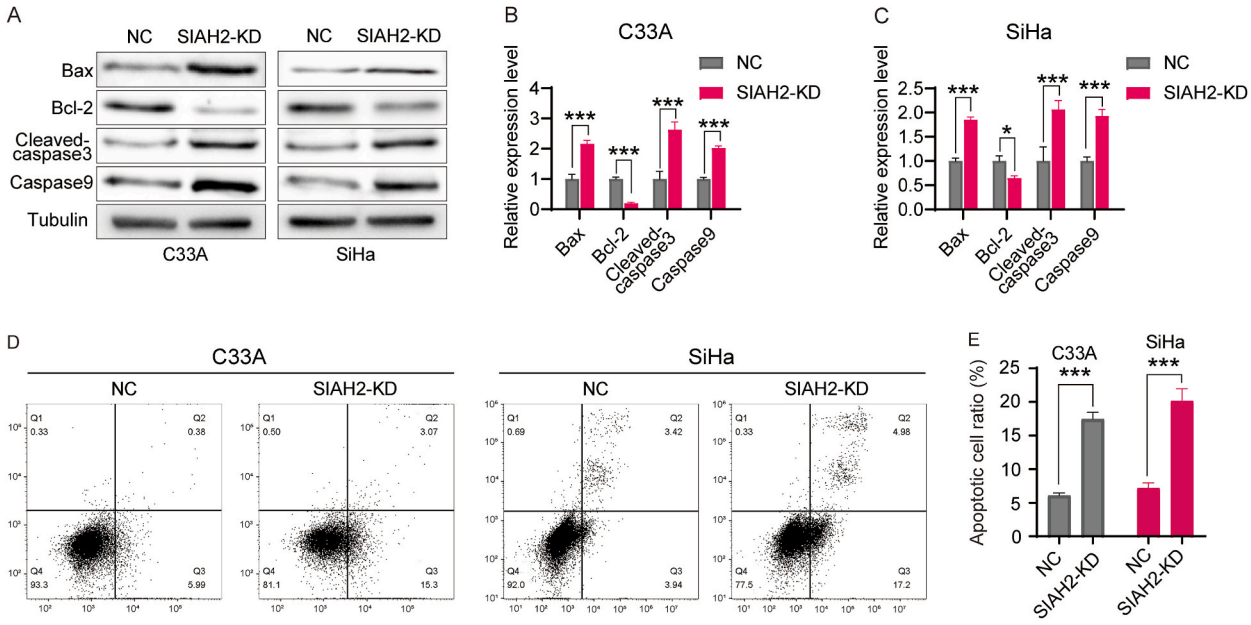

Fig. 3. SIAH2 knockdown activates the apoptosis signaling pathway.
(A) Western blot was performed to detected the expression of Bax, Bcl-2, Caspase3 and Caspase9. The original images of Western blot are listed in Supplementary Material 2. The relative expression levels of target proteins in C33A (B) and SiHa (C) cells were analyzed using ImageJ software. (D and E) The percentages of apoptotic cells were assayed using flow cytometry and Annexin V-FITC/PI double staining. *P < 0.05, **P < 0.01, ***P < 0.001.

the relative healing area was remarkably inhibited in SIAH2 knockdown C33A cells 48 h after scratch compared with the control cells (Fig. 2C). In SiHa cells, the relative healing area of SIAH2 knockdown group declined significantly 24 h and 48 h after scratch (Fig. 2D). Subsequently, transwell assay was performed to further confirm the effect of SIAH2 on cell migration and invasion (Fig. 2E). As expected, SIAH2 knockdown remarkably decreased the migration and invasion ability of C33A cells (Fig. 2F). Furthermore, the migration and invasion abilities were also inhibited by SIAH2 knockdown in SiHa cells (Fig. 2G). Collectively, our data demonstrate that attenuation of SIAH2 expression inhibited the migration and invasion of cervical cancer cells.

3.3. SIAH2 knockdown activates the apoptosis signaling pathway

Then, the apoptosis related proteins were detected using Western blot to clarify the effect of SIAH2 knockout on apoptosis in cervical cancer cells (Fig. 3A). As shown in Fig. 3B and C, the expression levels of Bax, Cleaved-caspase3 and Caspase9 were significantly upregulated by SIAH2 knockdown, while the expression of Bcl-2 was significantly downregulated by SIAH2 knockdown. Furthermore, the results of Annexin V-FITC/PI double staining assay showed that the percentage of cells undergoing apoptosis was significantly increased after SIAH knockdown (Fig. 3D and E). These results indicated that SIAH2 knockdown activates the apoptosis signaling pathway in cervical cancer cells.

3.4. Knockdown of SIAH2 reverses EMT progression

To excavate the potential mechanism of SIAH2 in cervical cancer progression, we detected changes in expression of factors involved in tumor-associated signaling pathways or tumor-initiating events using Western blot. The cytological basis of tumor invasion and metastasis is the loss of epithelial cell polarity and intercellular connection, and the reconstruction of cytoskeleton, which increase the migration and mobility, and obtain the characteristics of mesenchymal cells, that is, epithelial mesenchymal transformation (EMT) [12].

To clarify the mechanism of SIAH2 in migration and invasion, we detected the key factors in EMT process using Western blot (Fig. 4A). As shown in Fig. 4B and C, the expression levels of N-cadherin, Vimentin and Snail1 was significantly downregulated by SIAH2 knockdown, while the expression of E-cadherin were significantly upregulated by SIAH2 knockdown. These data suggested that knockdown of SIAH2 could reverse EMT progression.

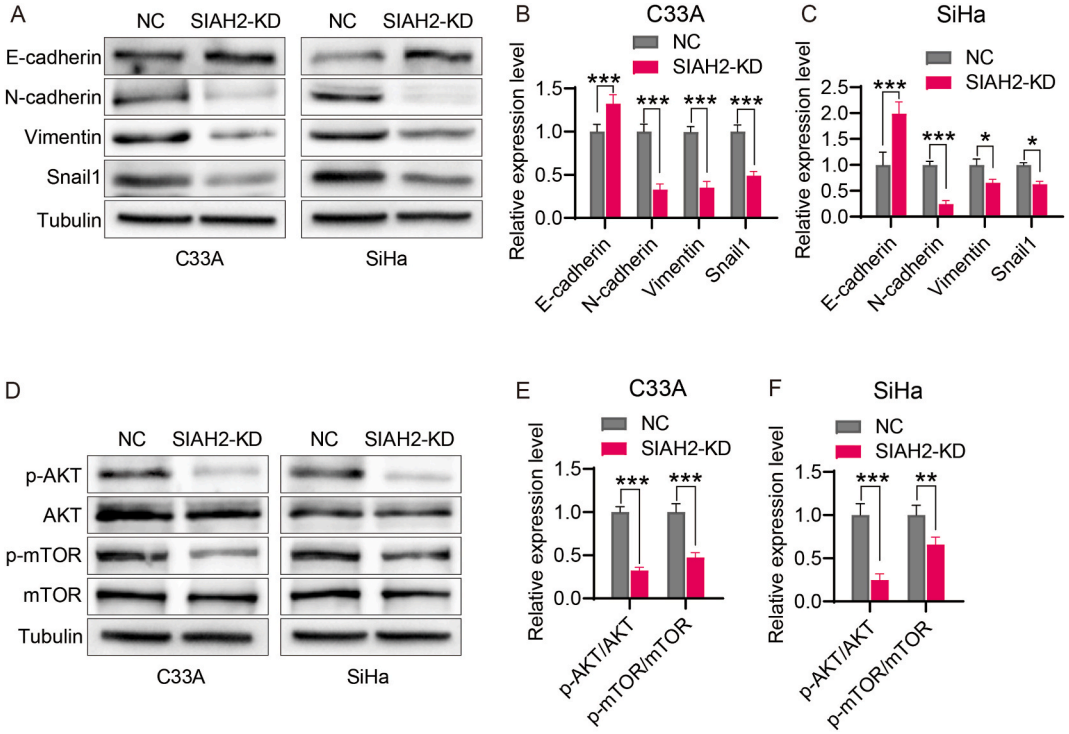

Fig. 4. Knockdown of SIAH2 reverses EMT progression.
(A) Western blot was performed to detected the expression of E-cadherin, N-cadherin, Vimentin and Snail1. The original images of Western blot are listed in Supplementary Material 3. The relative expression levels of EMT proteins in C33A (B) and SiHa (C) cells were analyzed using ImageJ software. (D) Western blot was performed to detected the expression of *p*-AKT, AKT, *p*-mTOR and mTOR. The original images of Western blot are listed in Supplementary Material 4. The relative expression levels of target proteins in C33A (E) and SiHa (F) cells were analyzed using ImageJ software. *P < 0.05, **P < 0.01, ***P < 0.001.

3.5. Knockdown of SIAH2 inhibits the activity of AKT/mTOR signaling pathway

AKT/mTOR signaling pathway is one of the most important signal transduction pathways in mammalian cells [13]. It inhibits apoptosis and promotes proliferation by affecting many downstream effectors. Here, we detected the expression of *p*-AKT, AKT,

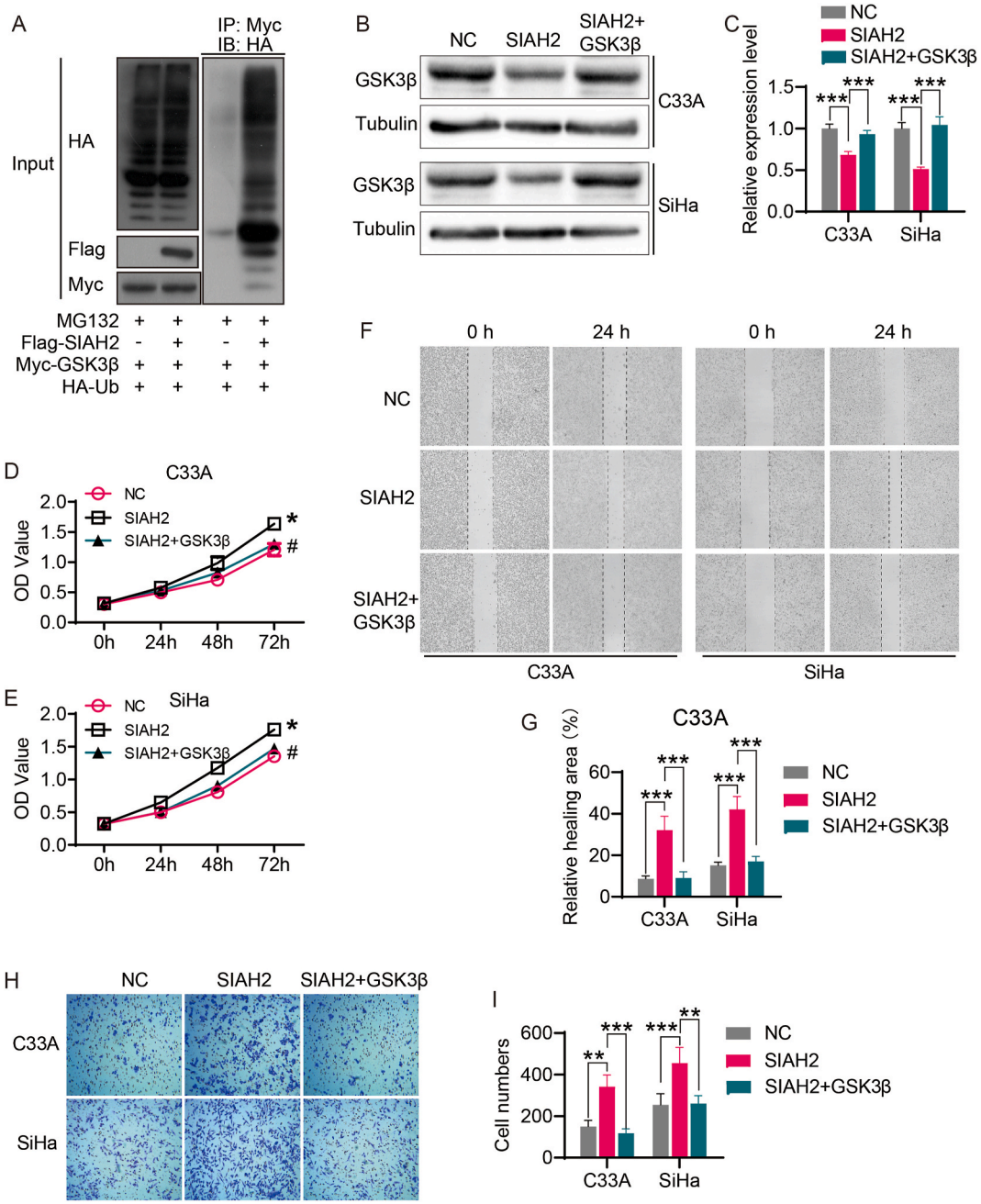

Fig. 5. GSK3β rescues the decrease in cell proliferation and invasion caused by SIAH2 overexpression.
(A) The indicated plasmids were transfected into the cells. Immunoprecipitation was performed to detect the ubiquitylation of GSK3β. The original images of Western blot are listed in Supplementary Material 5. (B and C) SIAH2 or GSK3β overexpression plasmids was transfected into C33A and SiHa. The expression of GSK3β was detected using Western blot. The proliferation of C33A (D) and SiHa (E) cells was detected using CCK8 assay. *P $<$ 0.05 vs. NC group; #P $<$ 0.05 vs. SIAH2 group. (F) Wound healing assay was performed to detect the migration of C33A and SiHa cells. (G) The relative healing area of C33A and SiHa cells was analyzed after 48 h of scratch. (H) Transwell assay was performed to further confirm the effect of SIAH2 on cell migration and invasion. (I) The migrated or invasive cell number of C33A and SiHa cells was counted in 3 random fields. **P $<$ 0.01, ***P $<$ 0.001.

p-mTOR and mTOR using Western blot (Fig. 4D). The phosphorylation levels of AKT and mTOR declined significantly after the transfection of SIAH2 siRNA both in C33A and SiHa cells (Fig. 4E and F). SIAH2 might participate in the regulation of cell proliferation and apoptosis through AKT signaling pathway.

3.6. GSK3β rescues the increase in cell proliferation and invasion caused by SIAH2 overexpression

In addition, we verified the interaction between SIAH2 and GSK3β using immunoprecipitation. As shown in Fig. 5A, Overexpression of SIAH2 significantly promoted the ubiquitylation of GSK3β. These data proved that SIAH2 could induce GSK3β degradation by mediating its ubiquitylation.

Then, we rescued GSK3β in SIAH2 overexpression cells through the transfection of GSK3β overexpression plasmid (Fig. 5B and C). According to CCK8 results, proliferation ability enhanced by SIAH2 is inhibited by GSK3β overexpression both in C33A and SiHa cells (Fig. 5D and E). Wound healing assay proved that the relative healing area increased in the SIAH2 overexpressed cells, and GSK3β overexpression inhibited this promotion (Fig. 5F and G). GSK3β overexpression repressed the migration promoting effect of SIAH2. Subsequently, transwell assay showed that the invasive cells number also increased in the SIAH2 overexpression group, and decreased significantly after the transfection of GSK3β overexpression plasmid. GSK3β inhibited the migration promoting effect of SIAH2 in cervical cancer cells.

3.7. SIAH2 is highly expressed in cervical cancer tissues

Subsequently, of the specific SIAH2 expression levels were examined in inflammatory cervical lesions, precancerous lesions and cervical cancer tissues by using IHC. Little staining for SIAH2-specific antibody was detected in 6 cases of inflamed cervical tissues, meaning that no SIAH2 expression was detected (Fig. 6A). Slight staining (beige) for SIAH2 antibody meaning low SIAH2 expression was detected in 69 precancerous cervical tissues, whereas strong staining (dark brown) for SIAH2 antibody meaning high SIAH2 expression was detected in 55 cervical cancer tissues (Fig. 6A). The data statistics and analysis showed that specific expression of SIAH2 was appeared in precancerous and cervical cancer tissues compared to inflammatory tissues, and the more SIAH2 was expressed as the degree of cancer progressed (**Table 1**). SIAH2 was significantly highly expressed in cervical cancer tissues (44/55, 80 %) compared with precancerous tissues (18/69, 26.1 %) (**Table 1**). These results suggested that the specific expression of SIAH2 was closely related to the abnormal proliferation of cervical epithelial cells. In addition, the expression level of SIAH2 in cervical cancer

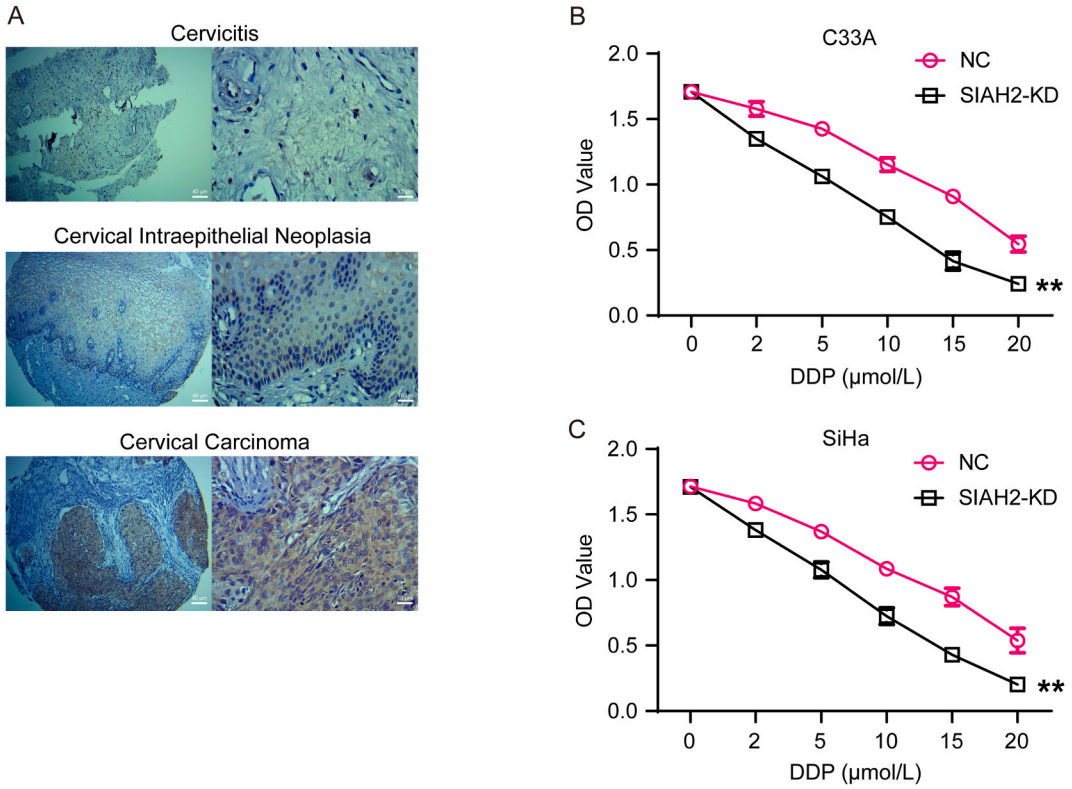

Fig. 6. SIAH2 is highly expressed in cervical cancer tissues
(A) The IHC stain of SIAH2 in the tissues from cervicitis, cervical intraepithelial neoplasia, cervical carcinoma. Left: 100x; Right: 400x. The survival of SIAH2 knockdown C33A (B) and SiHa (C) cells under different concentrations of cisplatin treatment was detected by CCK-8 assay. **P < 0.01.

Table 1
SIAH expression in cervical carcinoma compared with cervical intraepithelial neoplasia and cervicitis tissues.

Group	n	SIAH2 expression		P
		Low (n%)	High (n%)	
cervical carcinoma	55	11 (20)	44 (80)	0.0001
cervical intraepithelial neoplasia	69	51 (73.9)	18 (26.1)	
cervicitis	6	6 (100)	0 (0)	

tissues was significantly correlated with the degree of cancer differentiation, and cervical cancer tissues with greater levels of SIAH2 expression were less differentiated, but not with patient age, tumor diameter, lymph node metastasis, and depth of stromal invasion (**Table 2**).

3.8. SIAH2 knockdown sensitizes cells to cisplatin

The role of chemotherapy in the treatment of cervical cancer has attracted more and more attention, and it is mainly applied in radiotherapy for sensitization. In addition, there are preoperative neoadjuvant chemotherapy and palliative treatment for patients with advanced distant metastasis and recurrence. The preferred chemotherapeutic agent is cisplatin. In view of the reported role of SIAH2 in the chemosensitivity of cancer cells, we examined the survival of SIAH2 knockdown C33A and SiHa cells under cisplatin treatment. As shown in Fig. 6B and C, different concentrations of DDP treatment (24 h) significantly impaired the survival of C33A and SiHa cells. Moreover, compared with the NC group, SIAH2 knockdown cells were more sensitive to DDP, and fewer cells survived in the same concentration of DDP treatment.

4. Discussion

SIAH2 is an E3 ubiquitin ligase that maps to 3q25 and consists of 2 exons and 1 intron of approximately 9.8 kb. It is expressed in proliferating cells, but absent in non-proliferating cells. SIAH2 has been found to be expressed in various malignant tumors and cell lines such as pancreatic cancer, breast cancer, and lung cancer [14,15,16]. The expression of SIAH2 enhances with the increase of tumor malignancy [17]. It expressed in some benign tumors or tissues with active proliferation, such as colorectal adenomas and embryonic kidney cell lines, but hardly expressed in normal tissues such as colorectal mucosal epithelium and alveolar epithelium [17]. Therefore, it is speculated that SIAH2 exerts the biological effects as an oncogene. In this study, we found that SIAH2 was weakly expressed in inflamed cervical tissues, strongly highly expressed in cervical cancer tissues, and relatively little expressed in precancerous cervical tissues, and SIAH2 expression in cervical cancer tissues was significantly correlated with the degree of cancer differentiation.

Here, we confirmed that knockdown of SIAH2 was able to inhibit the proliferation, migration and invasion of cervical cancer cells in an *in vitro* cell model. Previous studies have confirmed that knockdown of SIAH2 has a tumor suppressor effect in a variety of tumors. In CWR22Rv1 prostate cancer cells, SIAH2 inhibition decreased intracellular androgen levels while inhibiting cell growth *in vitro* and in orthotopic prostate tumors [18]. It was also found that transfection of SIAH2-shRNA to inhibit the expression of SIAH2 could reduce the expression level of *p*-ERK, inhibit the growth, and promote apoptosis in four lung cancer cell lines [19]. In addition, this study detected the correlation of SIAH2 expression with the EMT process and activation of Akt/mTOR signaling pathway in cervical cancer cells. Although it has been reported that SIAH2 expression promotes migration and invasion in colorectal, gastric, hepatocellular, and breast cancers [9,10,20,21], to the best of our knowledge, no study has reported that SIAH2 can affect the EMT process in cancer cells,

Table 2
SIAH2 expression associated with the clinicopathological parameters in cervical carcinoma.

Clinicopathological Parameters	n	SIAH2 expression		P
		Low (n%)	High (n%)	
Age (years)				
≤50	25	5 (20.0)	20 (80.0)	0.735
>50	30	6 (25.0)	24 (75.0)	
Tumor diameter (cm)				
≤4.5	20	3 (13.6)	19 (86.4)	0.536
>4.5	25	8 (24.2)	25 (75.6)	
Lymph node metastasis				
Yes	17	2 (11.8)	15 (88.2)	0.511
NO	38	9 (23.7)	29 (76.3)	
Degree of tumor differentiation				
Low	35	3 (8.6)	32 (91.4)	0.014
Middle + high	20	8 (40)	12 (60)	
Depth Of Stromal Invasion				
≤1/2	14	11 (78.6)	3 (21.4)	0.816
>1/2	41	33 (80.5)	8 (19.5)	

and the specific mechanism of the effect still needs to be further explored. In colon cancer, SIAH2 has also been reported to activate the PI3K/Akt signaling pathway [9]. Our results again clarify that SIAH2 siRNA has anticancer effects.

It seems logical that SIAH2 knockdown cervical cancer cells are more sensitive to cisplatin treatment and less likely to survive cisplatin treatment. In addition to cisplatin, other studies have reported that SIAH2 knockdown can increase the sensitivity of cancer cells to other chemotherapeutic agents [6,22,23]. SIAH2 knockdown can increase the response of breast cancer cells to adriamycin and paclitaxel [6,22,23]. All these results suggest that the combination of drugs targeting SIAH2 would be beneficial for cancer treatment.

SIAH2 as an E3 ligase can catalyze the ubiquitination of a variety of substrates, thereby mediating the functional regulation of tumor cells [8,24–26]. SIAH2 degrades nuclear respiratory factor 1 by mediating ubiquitination on lysine 230 and spatially downregulates mitochondrial gene expression, thereby enhancing the Warburg effect and metabolic reprogramming, and remodeling the tumor microenvironment to maintain and develop tumors [27]. Under hypoxia, the activity of SIAH2 enhances, which increases the degradation of substrate PHD3 and the expression of HIF-1α, and initiates the hyperactivation of its downstream target genes, including VEGF, c-Met and CXCR4, thereby promoting tumor proliferation and metastasis [28]. In the present research, we confirmed that GSK3β is a ubiquitinated target of SIAH2 in cervical cancer cells by ubiquitination assays, and SIAH2 induces its degradation by mediating GSK3β ubiquitylation. As the main serine/threonine family kinase in cells, GSK3β activity is closely related to the occurrence and development of various diseases [29,30]. Through diversified regulation modes, GSK3β is involved in multiple signal transduction pathways related to tumor formation, such as Wnt/β-catenin, NF-κB, etc., thereby regulating the growth, proliferation and apoptosis of tumor cells [31–33].

Subsequently, rescue experiments further clarified the fact that GSK3β acted as a functional target of SIAH2. GSK3β overexpression could rescue the increase in cell proliferation and invasion caused by SIAH2 knockdown. However, since GSK3β mediated regulation of tumor progression is bidirectional, GSK3β based cancer treatment strategies are controversial [33]. In fact, the insulation mechanism of biology can make the different effects of GSK3β not interfere with each other in the regulation of numerous signaling pathways, thereby determining cell fate. The specific strategies will become the focus of GSK3β clinical applications.

Limitations of this study include the lack of *in vivo* experiments as well as *in vitro* cellular experiments with SIAH2 overexpression.

5. Conclusion

In conclusion, SIAH2 was specifically expressed during cervical carcinogenesis, and was closely related to the abnormal proliferation of cervical epithelial cells. Knockdown of SIAH2 expression in human cervical cancer cells *in vitro* inhibited cell proliferation, migration and invasion, induced apoptosis and increased cell sensitivity to cisplatin. Mechanistically, SIAH2 induced ubiquitylation degradation of GSK3β, which regulated cervical cancer progression. Targeting SIAH2 may be beneficial for the treatment of cervical cancer.

Ethical statements

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Review Committee of Shandong University Affiliated Shandong Provincial Third Hospital (No. KYLL-2023015). Informed consent was obtained from all women before surgery.

Availability of data and material

The data supporting the conclusions of this paper are included within the manuscript.

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CRediT authorship contribution statement

Li-ping Jing: Writing – review & editing, Writing – original draft, Visualization, Validation. **Meng Li:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Xi-yan Xia:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Xin Zheng:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Jia-yu Chen:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Jing He:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Xue-wei Zhuang:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31487.

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