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CAF-1/p150 promotes cell proliferation, migration, invasion and predicts a poor prognosis in patients with cervical cancer

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Abstract. Cervical cancer is one of the most common malignancies among women worldwide that exhibits high morbidity and mortality rates. Thus, the discovery of novel molecules and targets for cervical cancer diagnosis and treatment is critical. The present study aimed to investigate the role of the chromatin assembly factor (CAF)-1 subunit, CAF-1/p150 on cervical cancer cell proliferation, migration and invasion. Immunohistochemical analysis was used to detect the CAF-1/p150 expression in cervical cancer tissues and to analyze the association between CAF-1/p150 expression and the prognosis of patients with cervical cancer. In addition, colony formation, wound healing and Transwell assays were used to assess the function of CAF-1/p150 in cervical cancer cells. The results demonstrated that CAF-1/p150 was expressed in both normal and cervical cancer tissues. CAF-1/p150 protein expression was localized in the cell nuclei and was highly expressed in cervical cancer tissues. Furthermore, high CAF-1/p150 expression was significantly associated with FIGO stage, local recurrence, distant metastasis and a shorter overall survival time of patients with cervical cancer. CAF-1/p150 knockdown attenuated the anchorage-independent proliferation, migration and invasion of Hela and SiHa cervical cancer cells *in vitro*. Taken together, the results of the present study confirmed the involvement of CAF-1/p150 in the progression of cervical cancer, and validated its use as a poor prognostic indicator in patients with cervical cancer.

Introduction

Cervical cancer is a fast-growing malignancy (1). Population statistics in 2018 reported that cervical cancer ranks fourth for both incidence and mortality for different types of cancer among women worldwide (2). A total of 570,000 new cases are diagnosed and 311,000 cervical cancer-associated mortalities occur globally each year (2). Despite notable improvements in early screening, effective diagnosis of cervical cancer remains difficult, with a 5-year survival rate of 40-60% (3,4). This is due to the fact that recurrence and metastasis occur in ~30% of patients following treatment (3). The molecular mechanisms involved in the progression of cervical cancer remain unclear. Thus, the discovery of novel molecules and targets for cervical cancer diagnosis and treatment is critical.

Chromatin assembly factor (CAF)-1 is a heterologous complex purified from 293T cells and contains three subunits (p150, p60 and p48), according to molecular weight (4). These subunits can form larger structures and directly bind to the acetylated forms of histones H3 and H4 to promote the assembly of histone proteins on newly synthesized DNA (5). CAF-1 promotes nucleosome assembly on DNA undergoing replication and is involved in the DNA repair process (6). CAF-1 expression is absent or very low in cells that are in the resting state and is highly expressed in proliferating cells (6). CAF-1 expression is associated with the proliferative state of cells and has previously been validated as a useful proliferation marker (7). In the mitotic phase of the cell cycle, CAF-1 accumulates in the nucleolus and stabilizes the heterochromatin structure during replication (8).

It has been demonstrated that CAF-1 can be used to predict the clinical outcome of patients with cancer (9). CAF-1 protein is aberrantly expressed in different tumor tissues and its high expression is significantly associated with high histological grade in breast and renal cell carcinomas, and with advanced disease stage in endometrial and renal carcinomas (10,11). It has been reported that CAF-1 expression in cervical cancer tissues is positively associated with Ki-67 expression and negatively associated with the overall survival (OS) time of patients (12). OS defined as the time from randomization to death from any cause, is a direct measure of clinical benefit to a patient. Notably, the OS time of patients with cervical cancer, with low CAF-1 expression, is prolonged (12). This suggests that high CAF-1 expression levels may be used as a predictor of adverse clinical outcomes in cervical carcinomas. However, the clinical significance of p150 expression in cervical cancer tissues, and its role in cervical cancer cells have not yet been fully investigated.

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In the present study, immunohistochemical analysis was used to detect the CAF-1/p150 expression in cervical cancer tissues and to investigate the clinical significance of CAF-1/p150 in cervical cancer. An *in vitro* cell culture model including HeLa and SiHa cervical cancer cell lines was used to silence CAF-1/p150 expression and further investigate its function in cervical cancer.

Materials and methods

Reagents. The recombinant anti-CAF-1/p150 antibody used for immunohistochemistry (IHC; cat. no. ab109442) and western blot (cat. no. ab126625) analyses was purchased from Abcam. The anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000; cat. no. BS10043) was purchased from Bioworld Technology, Inc. The biotin-labeled anti-rabbit secondary antibody (cat. no. A0277, 1:1,000) was purchased from Beyotime Institute of Biotechnology, Inc. Matrigel (cat. no. 356234) was purchased from BD Biosciences. The two-step immunohistochemical staining detection kit (cat. no. PV-9000) and 3'-diaminobenzidine (DAB) substrate were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. The hematoxylin and eosin (H&E) staining kit (cat. no. G1121) was purchased from Beijing Solarbio Science & Technology Co., Ltd.

Patients and specimens. Paraffin specimens were collected from patients with cervical cancer following biopsy or surgical resection at the Zunyi Medical College Affiliated Hospital (Zunyi, China) between January 2013 and August 2014. A total of 214 cases met the screening criteria, and 80 cases who did not undergo any anti-tumor treatment, including surgery, radiotherapy and chemotherapy, biological immunotherapy and traditional Chinese medical treatment, were enrolled onto the present study (patient age range, 29-72; mean age, 46.2 years). The clinicopathological characteristics of these patients are listed in Table I. Normal cervical tissue specimens (20 cases) were obtained from 20 patients (patient age range, 35-47; mean age, 41.6 years) who underwent total hysterectomy due to uterine fibroids, and the absence of cervical lesions was confirmed via pathological analysis by two pathologists (Zunyi Medical College Affiliated Hospital, China). The tumor stage of each patient was determined according to the International Federation of Gynecology and Obstetrics (FIGO, 2009) (13). The present study was approved by the Human Ethics Committee of Zunyi Medical College Affiliated Hospital [Zunyi, China, approval no. ZMC2012(841)]. The experiments were performed in accordance with The Declaration of Helsinki and in agreement with Chinese legislation. Written informed consent was provided by all patients prior to the study start.

IHC. Tumor tissues were fixed in formalin (10% formaldehyde) for 48 h at 25˚C, embedded in paraffin and the samples were cut into 4-μm-thick sections, which were mounted onto slides for H&E staining or immunohistochemical analysis of CAF-1/p150 expression. Briefly, the tissue sections were deparaffinized by heating at 60˚C for 2 h, washed three times with xylene (for 5 min each) and rehydrated in a descending ethanol series (100, 95, 90, 80 and 70% ethanol for 5 min each). Antigen retrieval was achieved following incubation in 10 mM sodium citrate (Sigma-Aldrich; Merck KGaA) buffer (pH 6.0) for 15 min at 98˚C. Subsequently, tissue sections were incubated with 3% H_2O_2 for 20 min at 25˚C to inhibit endogenous peroxidase activity. The slides were blocked with 100% goat serum (Dako; Agilent Technologies, Inc.) for 30 min 25˚C and incubated with rabbit anti-human CAF-1/p150 antibody (1:100) overnight at 4˚C. Following the primary incubation, tissue sections were subsequently incubated with biotin-labeled anti-rabbit secondary antibody for 20 min and streptavidin-HRP for 2 min at 25˚C. The slides were stained with DAB substrate for 15 sec at 25˚C and counterstained with hematoxylin for 2 min at 25˚C. Negative controls were incubated with pre-immune serum instead of the antibody overnight at 4˚C.

CAF-1/p150 expression was evaluated by 2 pathologists above mentioned in a double-blind experimental design. The staining intensity score was calculated as follows: 0, completely absent; 1, light yellow; 2, light brown and 3, dark brown. The area occupied by positive cells score was estimated as follows: 0 points, ≤10%; 1 point, 11-25%; 2 points, 26-50% and 3 points, >50%. The area occupied by positive cells score is the average number of positive cells in 5 randomly selected areas. The total score was calculated as the sum of the staining intensity score and the area occupied by positive cells score, as follows: 0 points, (-); 1-2 points, (+); 3-4 points, (++) and 5-6 points, (+++). (-) and (+) are defined as low expression, and (++) and (+++) are defined as high expression. The cut-off value is ≥3.0. Images were captured using a CKX41 inverted light microscope (magnification, x100) and NIS-Elements F3.0 acquisition software.

Follow-up information. All patients received standardized treatment following diagnosis. Routine telephone follow-up was performed every 3-6 months for the first 2 years and every 6-12 months for the next 3-5 years from January 2013 to April 2016. The total duration of follow-up was 40 months. The patients were assessed for local recurrence, distant metastasis and OS. Local recurrence and distant metastasis were confirmed via pathological analysis. OS was defined as the first day of treatment until mortality caused by cervical cancer or the last date of follow-up. A total of 80 patients were followed up for 8-40 months. The median follow-up time was 25 months. A total of 16 patients exhibited local recurrence, 12 patients presented with distant metastasis and 9 patients did not survive due to cervical cancer. The OS time of patients with high CAF-1/p150 expression was between 8-37 months, whereas the OS time of patients with low CAF-1/p150 expression was between 10-40 months.

Cell lines and cell culture. The HeLa and SiHa cell lines were purchased from the State Key Laboratory of Biotherapy, Sichuan University (passage number, 5-20, Sichuan University, Sichuan, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum (CS), 100 U/ml penicillin and 100 μg/ml streptomycin (all purchased from Gibco; Thermo Fisher Scientific, Inc.), at 37˚C in 5% CO_2.

RNA interference. CAF-1/p150 small interfering (si)RNA (forward, 5'-GCAUGUGCAUCACCCAAUUTT-3' and

reverse, 5'-AAUUGGGUGAUGCACAUGCTT-3') and non-specific siRNA control (forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGAATT-3') were designed and synthesized by Shanghai GenePharma Co., Ltd., and transfected into the HeLa and SiHa cells using 25 pmol/5x10^5 cells Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h, according to the manufacturer's protocol. After transfection, the HeLa and SiHa cells were cultured in fresh medium for next 24 h and then used for the subsequent experiments.

Wound healing assay. Following transfection with siRNA, the HeLa and SiHa cells (2x10^4 cells/well) were seeded into 12-well plates and scratched using a 10-μl micropipette tip once they reached ~90% confluence. Cells were subsequently incubated with serum-free medium for 48 h at 37˚C in 5% CO_2. Images were captured at 0 and 48 h using a CKX41 inverted light microscope (Olympus Corporation; magnification, x100) and NIS-Elements F3.0 acquisition software (Olympus Corporation). The migration rate was calculated as the difference between the wound width at 0 and 48 h. The migration rate=($W_{0\ h}$-$W_{48\ h}$)/$W_{0\ h.}$ Data are presented as the fold-change relative to the non-specific siRNA control cells.

Transwell assay. A Matrigel-coated Transwell chamber system (8-μm pore size; EMD Millipore) was used to assess the migratory and invasive abilities of cervical cancer cells in the absence or presence of siRNA sequences. The Matrigel was precoated on the membrane at 37˚C for 30 min.

Following transfection with siRNA, the HeLa and SiHa cells were digested by 0.25% Trypsin-EDTA (Gibico, Thermo Fisher Scientific, Inc.) and plated in the upper chambers of Transwell plates in serum-free DMEM medium (5x10^4 cells/ml; 400 μl). DMEM Medium supplemented with 10% CS (600 μl) was added plated in the lower chambers. Following incubation for 24 h at 37˚C in 5% CO_2, cells that did not cross the membrane were removed using cotton swabs, while cells that successfully crossed the membrane were fixed with 100% methanol at 25˚C for 20 min and stained with H&E at 25˚C for 20 min. Images were captured using a CKX41 inverted light microscope (magnification, x100) and NIS-Elements F3.0 acquisition software.

Colony formation assay. The colony formation assay was performed using 0.7% agarose (Sigma-Aldrich; Merck KGaA) as the bottom layer in 6-well plates. A second layer containing agarose was also used. Following transfection with siRNA, the Hela and SiHa cells were digested and resuspended in DMEM medium containing 0.35% agarose, which was used as the upper layer (1,000 cells/well). The gel was solidified, and 1 ml of DMEM medium (containing 10% CS, 100 U/ml penicillin and 100 μg/ml streptomycin) was added into the plates to prevent drying. Cells were cultured in fresh DMEM medium (containing 10% CS, 100 U/ml penicillin and 100 μg/ml streptomycin) every 3 days. Following incubation for 30 days at 37˚C in 5% CO_2, cell colonies were stained with 0.005% crystal violet at 25˚C for 1 min and imaged using a D7200 digital color camera (Nikon Corporation). The number of colonies were analyzed using ImageJ software (National Institutes of Health). Colony formation was calculated as the number colonies formed in the siRNA group divided by the number of colonies in the non-specific siRNA group.

Western blotting. The HeLa and SiHa cells were lysed in modified RIPA buffer (Beyotime Institute of Biotechnology), containing Tris-HCl 50 mM, pH: 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 μg/ml Aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM Na_3VO_4, 1 mM NaF) and centrifuged at 13,000 x g for 30 min at 4˚C. Protein concentration was determined using a bicinchoninic acid protein assay. Equal amounts of protein (50 μg) were separated via 12% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (EMD Millipore). Membranes were blocked with 5% non-fat milk for 1 h at 37˚C and subsequently incubated with CAF-1/p150 antibody (1:5,000) overnight at 4˚C. Following the primary incubation, membranes were incubated with anti-rabbit secondary antibody for 1 h at 37˚C. Protein bands were detected using the Horseradish Peroxidase Color Development kit (cat. no. p0018s-1; Beyotime Institute of Biotechnology), visualized using a chemiluminescence imaging system (Thermo Fisher Scientific, Inc.) and evaluated using Image Lab software version 6.0 (Bio-Rad Laboratories).

Statistical analysis. Statistical analysis was performed using SPSS software (version 21.0; IBM Corp.) Data are presented as the mean ± standard deviation. *In vitro* experiments were repeated three times. Student's t-test (unpaired) was used to compare differences between two groups. χ^2 test (unpaired) was used to determine the association between CAF-1/p150 expression and clinicopathological characteristics of patients with cervical cancer. Univariate analysis by χ^2 test was used to the significant differences between CAF-1/p150 expression and variables listed in Table I. Student's t-test, χ^2 test and univariate analysis were performed using SPSS version 15.0 (SPSS Inc.). Kaplan-Meier survival analysis and the Renyi test were performed to determine patient survival according to CAF-1/p150 expression, using the program in SAS software. $P<0.05$ was considered to indicate a statistically significant difference.

Results

CAF-1/p150 expression in normal and cervical cancer tissues. IHC analysis of CAF-1/p150 expression in normal and cervical cancer tissues is presented in Fig. 1A (b) and (c), respectively. The negative control, without CAF-1/p150 antibody in cervical cancer tissues is presented in Fig. 1A (a). CAF-1/p150 protein expression was localized in the nuclei of normal and cervical cancer cells. Specifically, low CAF-1/p150 expression (+) was observed in 13/20 cases of normal cervical tissues and in 21/80 cases of cervical cancer tissues. Moderate CAF-1/p150 expression (++) was observed in 7/20 cases of normal cervical tissues and in 5/80 cases of cervical cancer tissues. High CAF-1/p150 expression (+++) was observed in 54/80 cases of cervical cancer tissues but not in normal cervical tissues. It was demonstrated that CAF-1/p150 staining intensity was stronger in cervical cancer tissues compared with normal cervical tissues. Furthermore, a statistically significant difference of IHC analysis in CAF-1/p150 expression which is represented

Table I. Association between CAF-1/p150 expression and clinicopathological characteristics of patients with cervical cancer (n=80).

Characteristic	Patient number, n	CAF-1/p150 expression		χ^2 value	P-value
		Low	High		
Age, years				2.064	0.151
<50	21	8	13		
≥50	59	13	46		
Tumor size, cm				1.214	0.271
<4	57	13	44		
≥4	23	8	15		
Pathology				0.231	0.631
Squamous cell carcinoma	70	19	51		
Adenocarcinoma	10	2	8		
Vascular invasion				0.195	0.659
No	58	16	42		
Yes	22	5	17		
Lymphatic metastasis				0.016	0.898
No	58	15	43		
Yes	22	6	16		
FIGO stage				5.230	0.022[a]
I-II	40	15	25		
III-IV	40	6	34		

[a]P<0.05. FIGO, International Federation of Gynecology and Obstetrics; CAF, chromatin assembly factor.

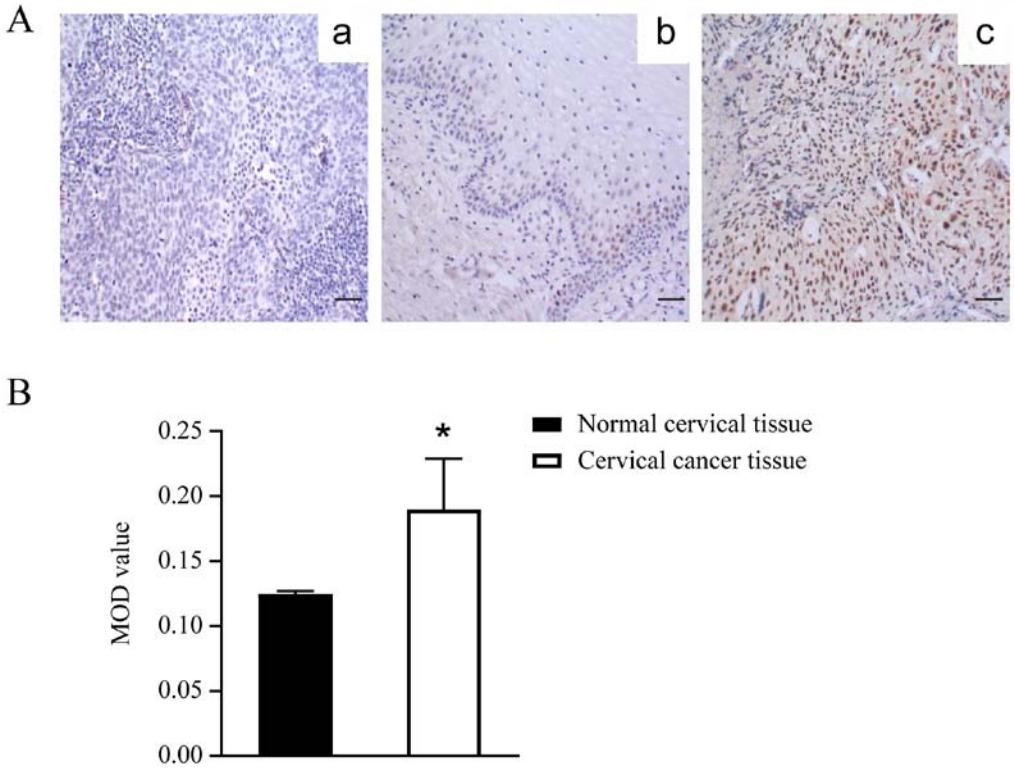

Figure 1. CAF-1/p150 expression in normal cervical and cervical cancer tissues. (A) Representative images of immunohistochemistry analysis of CAF-1/p150 expression in (a) the negative control, (b) normal cervical tissues and (c) cervical cancer tissues. Magnification, x100. Scale bar, 200 μm. (B) Statistical analysis of CAF-1/p150 expression in normal cervical and cervical cancer tissues. *P<0.05 vs. normal cervical tissues. CAF, chromatin assembly factor; MOD, mean optical density.

Table II. Association between CAF-1/p150 expression and clinical outcomes in patients with cervical cancer.

	CAF-1/p150 expression			
Follow-up	High	Low	χ^2 value	P-value
Local recurrence	13	3	4.091	0.043
Distant metastasis	10	2		
Death	8	1		

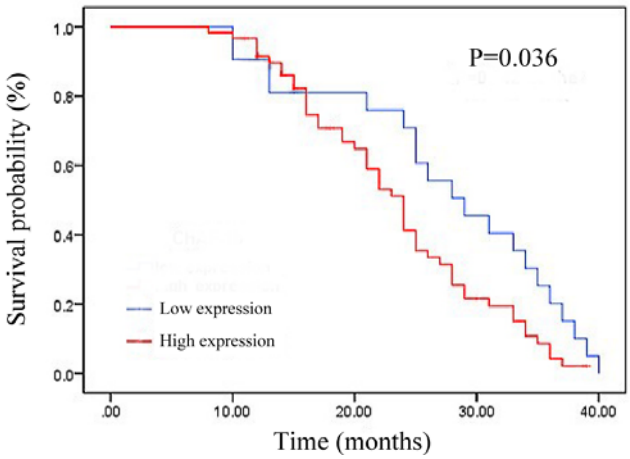

Figure 2. Kaplan-Meier survival analysis of patients with cervical cancer, according to CAF-1/p150 expression. CAF, chromatin assembly factor.

by mean optical density (MOD) was observed between normal cervical tissues (0.1247±0.0021) and cervical cancer tissues (0.1896±0.0392, P=0.001, Fig. 1A and B).

High CAF-1/p150 expression indicates poor clinical outcomes in patients with cervical cancer. Univariate analysis demonstrated no significant differences between CAF-1/p150 expression and age, tumor size, pathological type, vascular invasion and lymph node metastasis (Table I). Conversely, CAF-1/p150 expression was significantly associated with the different FIGO stages of patients with cervical cancer (χ^2=5.230; P=0.022 Table I). Among the 59 patients with high CAF-1/p150 expression, eight patients did not survive, 13 patients exhibited local recurrence and 10 patients presented with distant metastasis. Among the patients with low CAF-1/p150 expression, 1 patient did not survive, three patients presented with local recurrence and two patients exhibited distant metastasis. The results indicated that high CAF-1/p150 expression was significantly associated with local recurrence and distant metastasis (χ^2=4.091; P=0.043; Table II), suggesting that high CAF-1/p150 expression is indicative of a poor prognosis of patients with cervical cancer.

The association between CAF-1/p150 expression and OS time of patients with cervical cancer was assessed. The OS time of patients with high CAF-1/p150 expression was significantly lower than that of patients with low CAF-1/p150 expression (P=0.036; Fig. 2). These results suggest that high CAF-1/p150 expression is associated with a low OS time of patients with cervical cancer.

CAF-1/p150 knockdown impairs cervical cancer cell proliferation. In vitro analysis of CAF-1/p150 was performed using Hela and SiHa cervical cancer cells, and transfection efficiency was assessed via western blot analysis. Cells transfected with siRNA-CAF-1/p150 decreased the endogenous expression of this protein compared with the non-specific siRNA transfected cells (Fig. 3A). The colony formation assay demonstrated that the number of cell colonies significantly decreased in Hela and SiHa cells following transfection with siRNA-CAF-1/p150 (Fig. 3B).

CAF-1/p150 knockdown suppresses the migratory ability of cervical cancer cells. Wound healing and Transwell assays were performed to assess the cellular lateral and chemotactic migratory abilities of cervical cancer cells. The wound healing assay indicated that the number of migratory cells decreased to 55.6±6.9 and 44.8±7.1% in siRNA-CAF-1/p150 transfected Hela and SiHa cells, respectively (Fig. 4A). Furthermore, the number of migratory cells that penetrated the Transwell membrane significantly decreased to 45.2±7.7 and 51±5.4% in Hela and SiHa cells, respectively, following transfection with siRNA-CAF-1/p150 (Fig. 4B).

CAF-1/p150 knockdown suppresses the invasive ability of cervical cancer cells. Transwell plates pre-coated with Matrigel were used to assess the invasive ability of cervical cancer cells. Compared with the invasive cells in the non-specific siRNA transfected cells, the invasive cells in siRNA-CAF-1/p150 transfected HeLa and SiHa cells reduced to 32.6±3.1 and 39.7±8.0%, respectively (Fig. 5).

Discussion

In recent years, the early diagnosis of cervical cancer has notably improved with the development of specific screening methods (14). However, the existing screening methods of cervical cancer, including exfoliated cytology and high-risk HPV detection, lack sensitivity and specificity (15). Thus, it is necessary to identify novel prognostic markers that can describe the degree of malignancy and provide information regarding the occurrence and mortality of patients with cervical cancer. Furthermore, the identification of these molecules can aid the design of therapeutic agents for cervical cancer.

Previous studies have assessed the expression levels of CAF-1 subunits via IHC analysis (16-19). These findings may help improve the prognosis of different types of solid human malignancies. In the present study, IHC analysis of CAF-1/p150 expression was performed in 20 normal cervical tissue slices and 80 cervical cancer tissue slices. The results demonstrated that CAF-1/p150 expression was elevated in cervical cancer tissues compared with normal cervical tissues. Although three subunits (p150, p60 and p48) of CAF-1 have been identified, their association with the clinicopathological characteristics of the progression of cervical cancer requires further investigation. CAF-1/p60 expression is associated with the histological grading of breast cancer, cervical cancer and endometrial carcinoma,

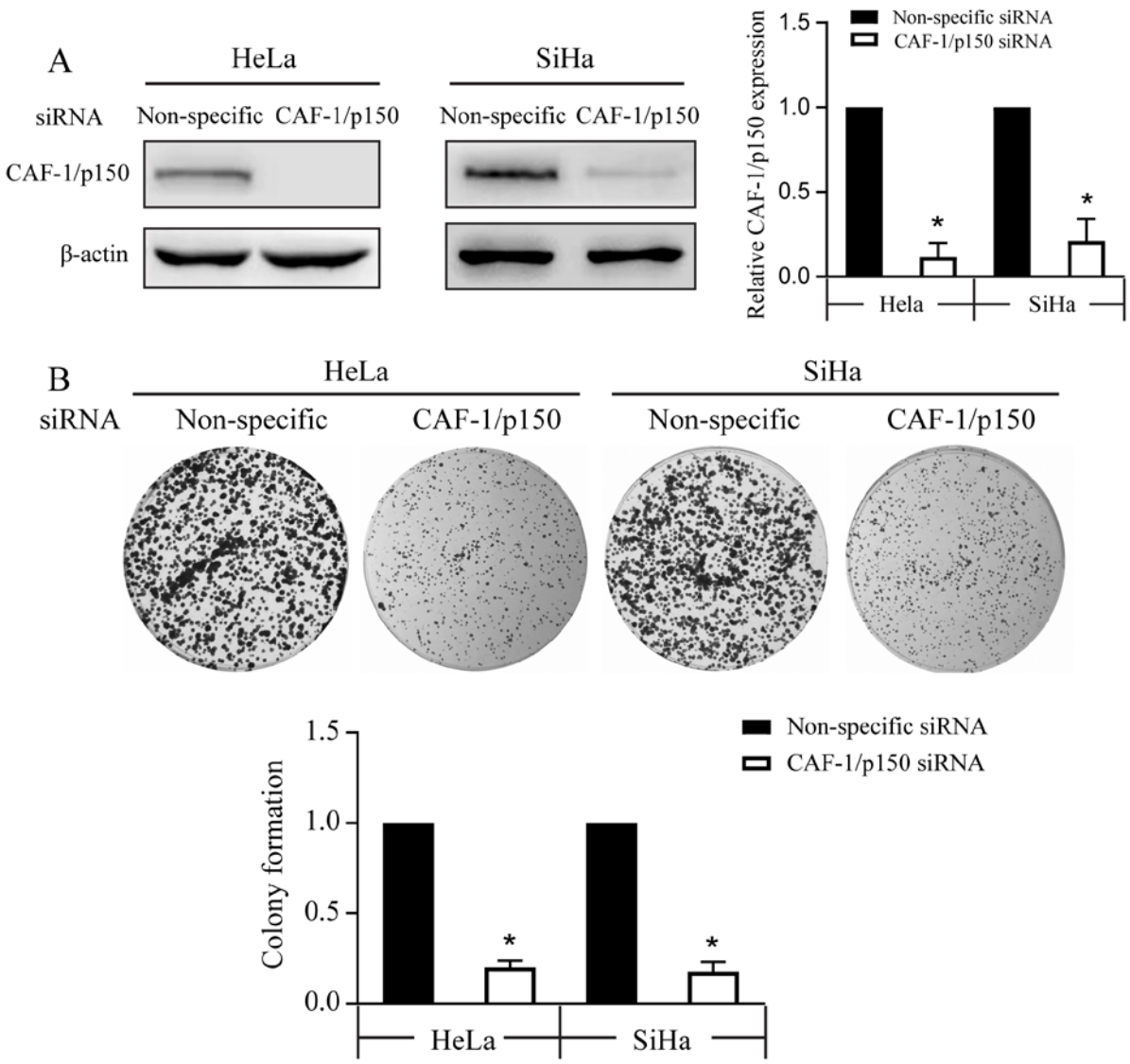

Figure 3. CAF-1/p150 knockdown impairs cervical cancer cell proliferation. (A) Transfection efficiency was assessed via western blot analysis. (B) Cell colony formation assay was performed and the results demonstrated that the total number of colonies decreased in cells transfected with siRNA-CAF-1/p150 compared with cells transfected with non-specific siRNA control. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. non-specific siRNA control. CAF, chromatin assembly factor; si, small interfering.

and with the clinical stage of endometrial carcinoma (12). In the present study, the association between CAF-1/p150 expression and prognosis of patients with cervical cancer was assessed. The results indicated that the expression levels of CAF-1/p150 protein in cervical cancer tissues were closely associated with the FIGO stage, whereas no significant associations were observed between CAF-1/p150 expression and age, tumor size, pathological type, vascular infiltration and lymph node metastasis. The CAF-1 subunit, p60 is considered a novel prognostic marker in oral squamous cell carcinoma, prostate cancer, salivary gland tumors and cutaneous melanoma (8,11). Overexpression of CAF-1/p60 is significantly associated with the biological aggressiveness of tumors and their metastatic phenotype (8,11,20). Similarly, the results of the present study demonstrated that cervical cancer cases with high CAF-1/p150 expression were significantly associated with tumor recurrence, metastasis and shorter OS time.

Previous studies have predominantly focused on the function of CAF-1 on cell proliferation and cell cycle distribution (9,21-23). CAF-1 is associated with maintaining appropriate nucleosome assembly during DNA synthesis and DNA repair (9). Among the three subunits, p48 is mainly responsible for acetylation/deacetylation of histones (21), whereas the p60 subunit acts specifically on cell replication (22) and the p150 subunit induces high activity by regulating the DNA double-strand break repair process (23). Thus, CAF-1/p150 exerts a key role in cell proliferation, most likely due to the high demand for DNA replication and chromatin formation.

CAF-1/p150 appears to function upstream of Ki-67, as it regulates the nucleolar localization and the peri-chromosomal layer accumulation of Ki-67 during interphase and mitosis (24). Consistent with previous findings, the results of the present study demonstrated that the anchorage-independent proliferative ability, which leads to colony formation in soft agar decreased when CAF-1/p150 protein was knocked down in the Hela and SiHa cervical cancer cell lines. Furthermore, the clinical observations from the cervical cancer samples suggested

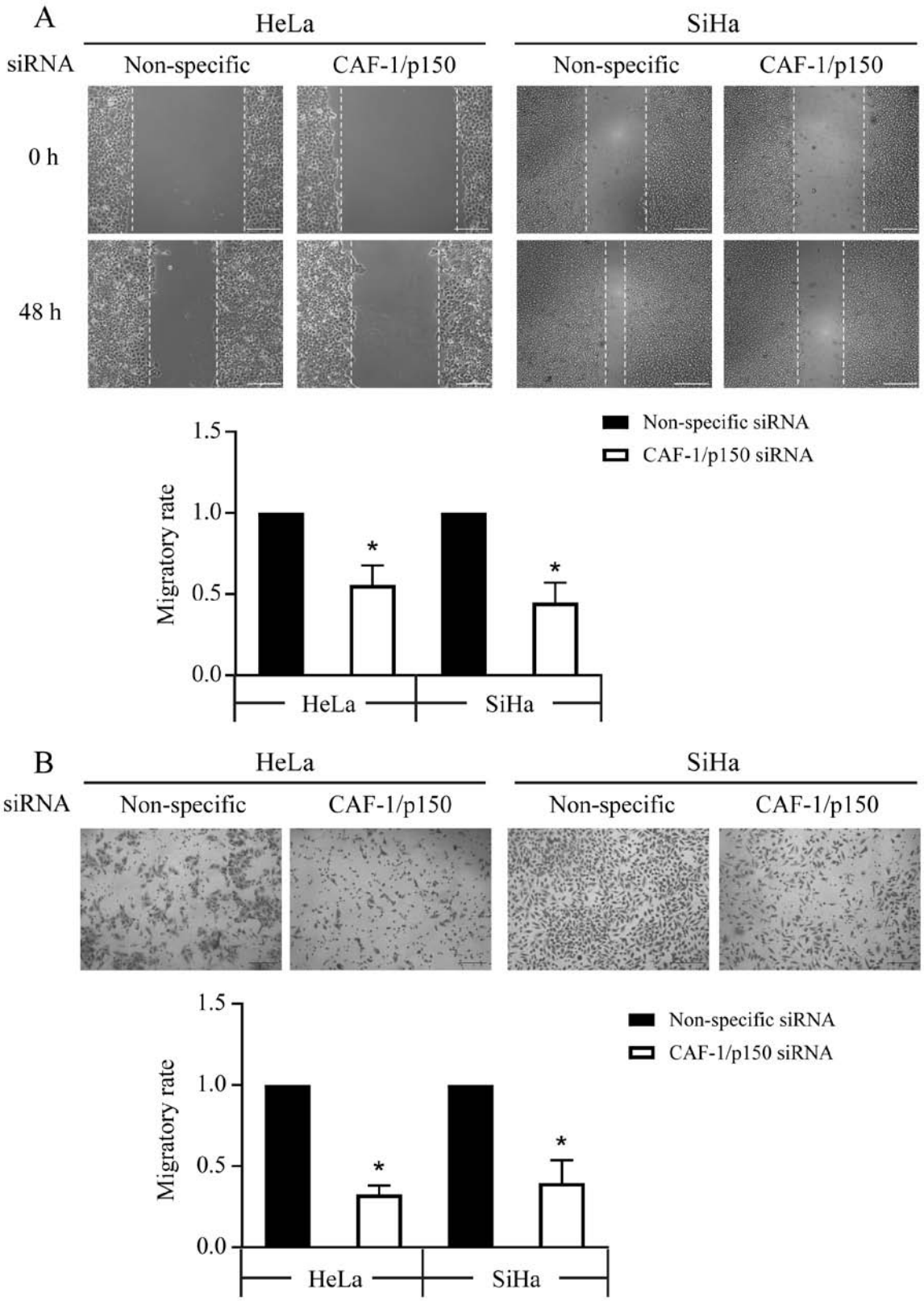

Figure 4. CAF-1/p150 knockdown suppresses the migratory ability of cervical cancer cells. (A) The cellular lateral migratory ability of cervical cancer cells was assessed via the wound healing assay. Magnification, x100; scale bar, 200 μm. (B) The cellular chemotactic migratory ability was assessed via the Transwell assay. Magnification, x100; scale bar, 200 μm. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. the non-specific siRNA control. CAF, chromatin assembly factor; si, small interfering.

that CAF-1/p150 exhibited additional functions beyond DNA replication. The speculation that this protein may possess a promoting role in driving metastasis of cervical cancer was confirmed through the use of cell line models. CAF-1/p150 knockdown suppressed cervical cell migration and invasion, which are the two necessary biological processes for tumor

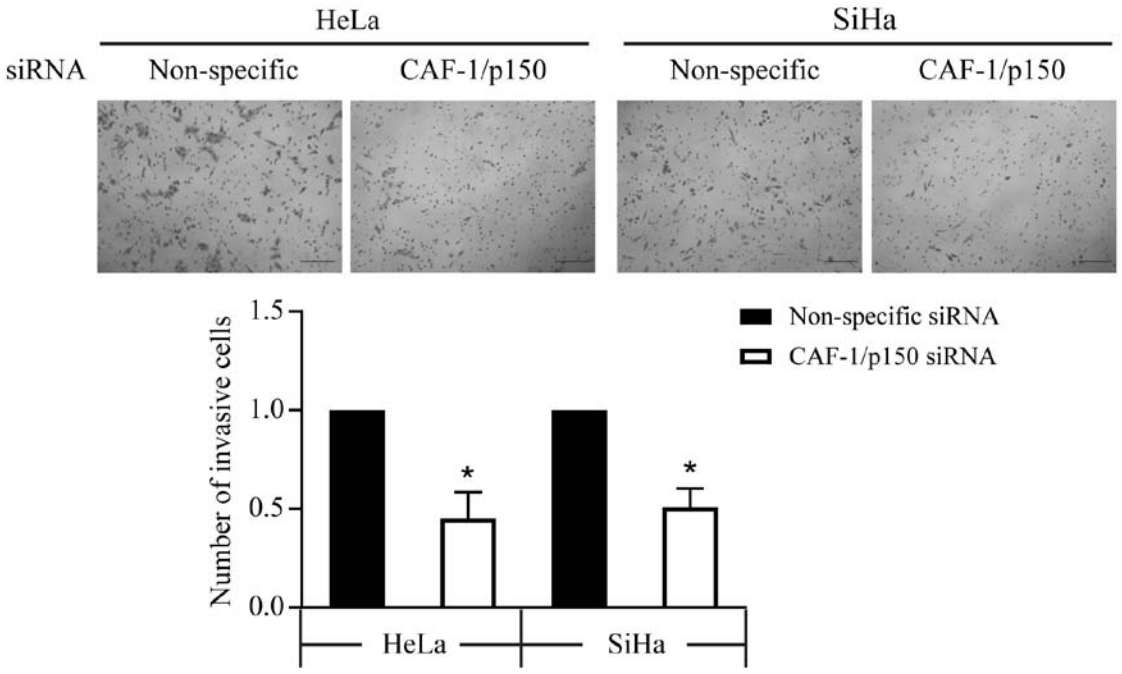

Figure 5. CAF-1/p150 knockdown suppresses the invasive ability of cervical cancer cells. Invasive cells that passed through the Transwell membrane were detected via hematoxylin and eosin staining. Magnification, x100; scale bar, 200 μm. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. non-specific siRNA control. CAF, chromatin assembly factor; si, small interfering.

metastasis (25). Thus, both *in vitro* and *in vivo* analyses confirmed that CAF-1/p150 exerted a positive function in cell proliferation, migration and invasion. However, further studies are required to determine the detailed molecular mechanisms by which CAF-1/p150 enhances cellular migration and invasion, as well as its downstream signaling members and it potential crosstalk with other molecular targets.

In conclusion, the results of the present study demonstrated that CAF-1/p150 expression abnormally increased in cervical cancer tissues compared with the corresponding normal cervical tissues. Furthermore, CAF-1/p150 expression was closely associated with the degree of cervical cancer malignancy and with a poor prognosis of patients with cervical cancer. CAF-1/p150 knockdown significantly suppressed cell proliferation, migration and invasion. Collectively, these results support the hypothesis that CAF-1/p150 is a promising candidate and a reliable prognostic marker for the identification of different types of human cancer with aggressive phenotypes.

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Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author upon reasonable request.

Authors' contributions

SY designed the present study, performed the literature analysis and drafted the initial manuscript. QL collected and analyzed the data. MC performed *in vitro* experiments. XL performed the statistical analysis and helped MC to perform the siRNA transfection. HZ designed and conceptualized the project and interpreted the data. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by the Human Ethics Committee of Zunyi Medical College Affiliated Hospital [Zunyi, Guizhou, China, approval no. ZMC2012(841)]. The experiments were performed in accordance with The Declaration of Helsinki and in agreement with Chinese legislation. Written informed consent was provided by all patients prior to the study start.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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