Elevated AKIP1 expression is associated with tumor invasion, shorter survival time and decreased chemosensitivity in endometrial carcinoma

AILI LI[1*], AIJING LI[2*], XIANGPENG GAO[3], TONGYAN ZHANG[1], ZHILING MA[1], YALIN XIAO[1] and FEI ZHAO[4]

[1]Department of Gynecology, Handan Central Hospital, Handan, Hebei 056001; [2]Department of Gynecology, Renqiu People's Hospital, Cangzhou, Hebei 062550; [3]School of Clinical Medicine, Graduate School of Hebei University, Baoding, Hebei 071002; [4]Department of Surgery, Handan Central Hospital, Handan, Hebei 056001, P.R. China

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Abstract. A-kinase-interacting protein 1 (AKIP1), as a recently discovered oncoprotein, promotes cell malignant behaviors in gynecological malignancies. To the best of our knowledge, no study reports its clinical value in patients with endometrial carcinoma. The present study aimed to explore the association between AKIP1 expression and clinical features and survival in patients with endometrial carcinoma, and to assess the effect of AKIP1 knockdown on the regulation of chemosensitivity *in vitro*. The tumor and adjacent tissue specimens from 101 patients with endometrial carcinoma were retrieved for AKIP1 protein expression analysis using an immunohistochemistry (IHC) assay. Meanwhile, specimens from 54 patients with endometrial carcinoma were analyzed for AKIP1 mRNA expression using reverse transcription-quantitative PCR. Furthermore, an *in vitro* experiment was conducted in the Ishikawa cell line to determine the effect of AKIP1 modification on the chemosensitivity of cisplatin and paclitaxel. AKIP1 IHC score (P<0.001) and mRNA expression levels (P<0.001) were increased in tumor tissues compared with those in adjacent tissues. Moreover, increased AKIP1 IHC score was associated with lymphovascular invasion (P=0.007), advanced International Federation of Gynecology and Obstetrics (FIGO) stage (P=0.002) and shorter overall survival (OS) time (P=0.035) in the patients with endometrial carcinoma. Meanwhile, upregulated AKIP1 mRNA expression levels were associated with lymphovascular invasion (P=0.020) and advanced FIGO stage (P=0.027) in the patients with endometrial carcinoma. Multivariate Cox regression showed that tumor AKIP1 protein expression (high vs. low) independently predicted a shorter OS time (P=0.036). Silencing of AKIP1 decreased Ishikawa cell viability when treated with 5, 10, 20 and 40 μM cisplatin (all P<0.05) and decreased the half maximal inhibitory concentration value of cisplatin (P=0.003), whereas its effect on paclitaxel chemosensitivity was less obvious. Overall, elevated AKIP1 expression was associated with tumor invasion, shorter survival time and decreased chemosensitivity in endometrial carcinoma.

Correspondence to: Dr Fei Zhao, Department of Surgery, Handan Central Hospital, 15 Zhonghua South Street, Hanshan, Handan, Hebei 056001, P.R. China
E-mail: zhifei4296166@163.com

*Contributed equally

Abbreviations: AKIP1, A-kinase-interacting protein 1; IHC, immunohistochemistry; FIGO, International Federation of Gynecology and Obstetrics; OS, overall survival; NF, nuclear factor; EMT, epithelial-to-mesenchymal transition

Key words: endometrial carcinoma, AKIP1, survival, clinicopathological features, chemosensitivity

Introduction

Endometrial carcinoma, as a common gynecological malignancy, displays increasing incidence rate globally (1,2). An average annual increase of 11.3% for endometrial carcinoma incidence was observed in South Africa between 2003 and 2012, and an increase was also observed in Brazil, America, Japan and other 24 countries/regions (2). Regarding the management of endometrial carcinoma, total hysterectomy and bilateral salpingo-oophorectomy (with or without adjuvant therapy) are recommended for affected patients who are suitable for surgery, while external beam radiation therapy and/or brachytherapy (with or without chemotherapy) is commonly applied for those patients who are not suitable for surgery (3,4). For those patients with endometrial carcinoma who meet the criteria for consideration of fertility-sparing options, hormonal therapy (continuous progestin-based therapy) may be considered as an alternative therapy option (4). Despite the appropriate management, patients with endometrial carcinoma may experience multiple recurrences (locoregional recurrence and extrapelvic recurrence), leading to a poor long-term outcome (5,6). Thus, identifying potential biomarkers to improve the prognosis for patients with endometrial carcinoma is critical and urgent.

A-kinase-interacting protein 1 (AKIP1) has been reported to serve as an oncogene in several solid tumors, especially

in gynecological malignancies (7,8). For instance, AKIP1 may promote cervical cancer cell invasion and migration through regulating nuclear factor (NF)-κB-induced epithelial-to-mesenchymal transition (EMT) (7). Moreover, AKIP1 can enhance cervical cancer cell proliferation and angiogenesis by upregulating CXC-chemokines (such as CXCL1, CXCL2 and CXCL8) (8). In the clinical field, AKIP1 has been identified as a potential prognostic biomarker for clear cell renal cell carcinoma, non-small cell lung cancer, colorectal cancer, hepatocellular carcinoma and cervical cancer (9-13). To the best of our knowledge, no study has explored the clinical role of AKIP1 in patients with endometrial carcinoma.

The present study collected 101 pairs of tumor tissue and adjacent tissue from patients with endometrial carcinoma in order to explore the associations between AKIP1 expression and clinicopathological features and prognosis. In addition, the present study further assessed the effect of AKIP1 on regulating chemosensitivity in an endometrial carcinoma cell line.

Materials and methods

Patients. The present study retrospectively analyzed the cases of 101 female patients with endometrial carcinoma who were treated by surgical resection at Handan Central Hospital (Handan, China) between January 2016 and January 2020. By reviewing their clinical data, the eligible patients were enrolled based on the following criteria: i) Histopathological diagnosis of endometrial carcinoma; ii) age >18 years; iii) treated by surgical resection; iv) surgically removed specimens, including tumor and tumor-adjacent tissues, were available and accessible; v) main clinical data and survival data were retrievable; and vi) no history of other malignancies before the diagnosis of endometrial carcinoma. Approval was acquired from the Institutional Review Board (IRB) of Handan Central Hospital (approval number, HDZXYY-Ethics-2021015) before the implementation of the study. The requirement for written informed consent was absolved by the IRB, as the study was performed based on post-operative patient samples and existing clinical data, which involved no risk to the patients themselves and did not affect the privacy of the patients.

Data acquisition. The main preoperative clinical features of the patients were obtained from medical records or outpatient records, and included age, menopausal status (pre-menopause or post-menopause), comorbidities [diabetes mellitus (DM) and hypertension], histological subtype (endometrioid carcinoma, serous endometrial carcinoma and clear cell endometrial carcinoma), myometrial invasion status, cervical invasion status, lymphovascular invasion status and International Federation of Gynecology and Obstetrics (FIGO) stage (14). Furthermore, the survival data of the patients was collated from the follow-up documents, and the overall survival (OS) time was determined.

Specimen acquisition. Tumor and tumor-adjacent tissue (2 cm from the tumor tissue) specimens (formalin-fixed using 4% paraformaldehyde for >24 h and paraffin-embedded and sliced into 4-μm sections) of all patients were acquired from the specimen repository in order to perform an immunohistochemistry (IHC) assay aimed at evaluating AKIP1 protein expression. Meanwhile, 54 of the 101 patients had fresh-frozen specimens that were immediately stored in liquid nitrogen following surgical removal (stored at -80˚C), which were also collected to determine the mRNA expression levels of AKIP1 by reverse transcription-quantitative PCR (RT-qPCR) assay.

IHC assay. The IHC staining was implemented as described in a previous study (15), with the AKIP1 Monoclonal Antibody as the primary antibody (cat. no. MA5-26998) and Goat anti-Mouse IgG (H+L) as the secondary antibody (cat. no. 31430) (both Invitrogen; Thermo Fisher Scientific, Inc.). The primary antibody was diluted 1:150 and the secondary antibody was diluted 1:20,000. The staining (10 min at room temperature) and counterstaining (5 min at room temperature) were performed using diaminobenzidine and hematoxylin, respectively. The IHC staining result was viewed on a light microscope, and the AKIP1 expression was evaluated using an IHC scoring method (16). In brief, five high-power fields (x200 magnification) were randomly selected in each slide, then the mean percentage of positively stained cells in five fields was calculated and scored as 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) or 4 (76-100%). Meanwhile, the staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate) or 3 (strong), and the mean staining intensity score of five fields was calculated. The final IHC score was obtained by multiplying the two scores. Using a cut-off score of 3, the AKIP1 protein expression was classified as low expression (IHC score ≤3) and high expression (IHC score >3).

RT-qPCR assay. After extraction of total RNA from the tumor tissues and adjacent tissues using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.), RT was conducted using iScript™ Reverse Transcription Supermix (Bio-Rad Laboratories, Inc.) according to the manufacturer's instructions. qPCR was performed using the QuantiNova SYBR Green PCR Kit (Qiagen GmbH). The following thermocycling conditions were applied: 95˚C for 2 min, followed by 40 cycles of 95˚C for 5 sec and 61˚C for 30 sec. The relative expression of AKIP1 was calculated using the $2^{-\Delta\Delta Cq}$ method, where GAPDH served as an internal reference (17). The forward and reserve primers were designed in line with a previous study (11). According to the median expression level in the tumor tissue, the AKIP1 mRNA expression was classified as low expression (up to and including the median level) and high expression (greater than the median level) for survival analysis.

In vitro experiment. Human endometrial carcinoma Ishikawa cells (Shanghai Enzyme Research Biotechnology Co., Ltd.) were cultured in DMEM (Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (both Gibco; Thermo Fisher Scientific, Inc.), in a 5% CO_2 atmosphere at 37˚C. The small interfering (si)RNA targeting AKIP1 (50 nM) (sense, 5'-GGAGGCAGC TATCAAATATTT-3' and antisense, 5'-ATATTTGATAGC TGCCTCCTT-3') and the corresponding negative control (NC) siRNA (50 nM) (sense, 5'-GAATTAATTAAAGAT GGCCCGTTGTACT-3' and antisense, 5'-TCATCGAAGTTA TAGGGATACATTACGTGATC-3') were purchased from Thermo Fisher Scientific, Inc., and separately transfected

into the $5x10^5$ Ishikawa cells using Lipofectamine® 3000 reagent (Thermo Fisher Scientific, Inc.) at 37˚C for 6 h. At 48 h post-transfection, the AKIP1 mRNA expression levels were detected. Subsequently, the Ishikawa cells were divided into three groups: Cells transfected with AKIP1 siRNA, cells transfected with NC siRNA and cells without transfection (used as a blank control), accordingly. Cisplatin and paclitaxel (both MilliporeSigma; Merck KGaA) were used for the chemosensitivity assay. The cisplatin was prepared at concentrations of 0, 5, 10, 20, 40 and 80 μM, and the paclitaxel was prepared at concentrations of 0, 0.5, 1, 2, 4 and 8 nM, according to previous studies (18-20). The cells in the three groups were respectively treated with the cisplatin and paclitaxel at the prepared concentrations for 48 h at 37˚C, and then the cell viability in each group was determined using Cell Counting Kit-8 reagent (Beyotime Institute of Biotechnology) for 2 h and detected at a wavelength of 450 nm.

Statistical analysis. SPSS 26.0 (IBM Corp.) was applied for data analysis, and GraphPad Prism 7.02 (GraphPad Software Inc.) was used for graph plotting. Quantitative data are presented as the mean ± standard deviation or median (interquartile range). Qualitative data are presented as n (%). The comparison of AKIP1 expression between tumor and adjacent tissues was performed using Wilcoxon's signed rank test. Receiver operating characteristic curve analysis was applied to evaluate the accuracy of AKIP1 expression for differentiating between different tissues. Associations between AKIP1 expression and clinical features were analyzed using Spearman's correlation (for ordered categorical variables) and Wilcoxon's rank sum test (for unordered categorical variables). OS was displayed in Kaplan-Meier curves and analyzed by log-rank test. Prognostic implications of variables were estimated using Cox proportional hazard model regression analysis. To further validate the correlation of AKIP1 with OS, a search for AKIP1 was performed in The Human Protein Atlas database (https://www.proteinatlas.org/ENSG00000130707-ASS1/pathology/endometrial+cancer), from which, AKIP1-related survival data derived from The Cancer Genome Atlas database (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) was obtained. In the *in vitro* experiment, AKIP1 mRNA expression was compared using one-way analysis of variance followed by Tukey's test. The cell viability between the AKIP1 siRNA group and the NC siRNA group was determined by unpaired t-test. The half maximal inhibitory concentration (IC_{50}) was estimated by Probit regression, and the mean IC_{50} between the AKIP1 siRNA group and the NC siRNA group was also determined by t-test. The experiments were all performed in triplicate. $P<0.05$ was used to indicate a statistically significant difference.

Results

Clinical features of patients with endometrial carcinoma. The mean age of the analyzed patients with endometrial carcinoma was 63.5±9.6 years (Table I). There were 15 (14.9%) and 86 (85.1%) patients with a pre-menopause and post-menopause status, respectively. Moreover, 67 (66.3%), 11 (10.9%), 16 (15.8%) and 7 (6.9%) patients were diagnosed with endometrioid carcinoma G1/G2, endometrioid carcinoma G3, serous endometrial carcinoma and clear cell endometrial carcinoma, respectively. Furthermore, 61 (60.4%), 14 (13.9%), 20 (19.8%) and 6 (5.9%) patients were graded as FIGO stage I, stage II, stage III and stage IV, respectively. The detailed clinical characteristics of the patients with endometrial carcinoma are listed in Table I.

Table I. Characteristics of patients with endometrial carcinoma (n=101).

Variables	Value
Mean age ± SD, years	63.5±9.6
Menopausal status, n (%)	
Pre-menopause	15 (14.9)
Post-menopause	86 (85.1)
Diabetes mellitus, n (%)	
No	73 (72.3)
Yes	28 (27.7)
Hypertension, n (%)	
No	50 (49.5)
Yes	51 (50.5)
Histological subtype, n (%)	
Endometrioid carcinoma G1/G2	67 (66.3)
Endometrioid carcinoma G3	11 (10.9)
Serous endometrial carcinoma	16 (15.8)
Clear cell endometrial carcinoma	7 (6.9)
Myometrial invasion ≥1/2, n (%)	
No	61 (60.4)
Yes	40 (39.6)
Cervical invasion, n (%)	
None or epithelial	76 (75.2)
Stromal	25 (24.8)
Lymphovascular invasion, n (%)	
No	75 (74.3)
Yes	26 (25.7)
FIGO stage, n (%)	
I	61 (60.4)
II	14 (13.9)
III	20 (19.8)
IV	6 (5.9)

SD, standard deviation; FIGO, International Federation of Gynecology and Obstetrics.

AKIP1 expression in patients with endometrial carcinoma. AKIP1 IHC score was increased in tumor tissue compared with that in adjacent tissue in the patients with endometrial carcinoma [median (IQR), 4.0 (3.0-6.0) vs. 2.0 (2.0-4.0); $P<0.001$; Fig. 1A and B]. Moreover, AKIP1 IHC score was able to differentiate tumor tissue from adjacent tissue, with an area under the curve (AUC) of 0.778 (95% CI, 0.715-0.842; Fig. 1C).

AKIP1 mRNA expression was also elevated in tumor tissues compared with that in adjacent tissues in the patients

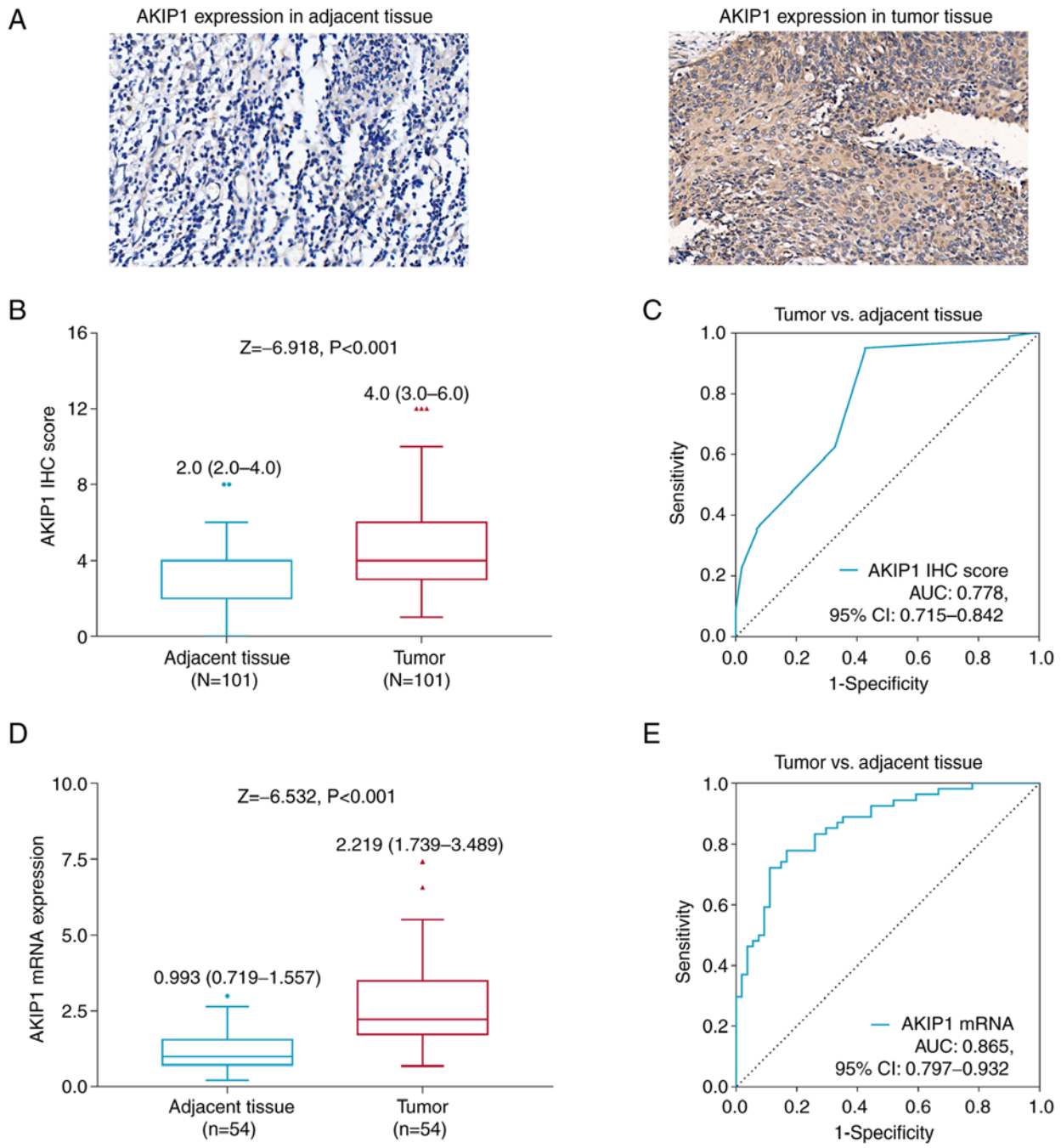

Figure 1. AKIP1 expression is increased in tumor tissue compared with that in adjacent tissue in patients with endometrial cancer. (A) Representative images of AKIP1 staining in adjacent and tumor tissues (magnification, x200). (B) Comparison of AKIP1 IHC score between adjacent and tumor tissues [data are presented as median (IQR)]. (C) ROC curve analysis of AKIP1 IHC score in differentiating tumor tissue from adjacent tissue. (D) Comparison of AKIP1 mRNA expression between adjacent and tumor tissues [data are presented as median (IQR)]. (E) ROC curve analysis of AKIP1 mRNA expression in differentiating tumor tissue from adjacent tissue. AKIP1, A-kinase-interacting protein 1; IHC, immunohistochemistry; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; IQR, interquartile range

with endometrial carcinoma [median (IQR), 2.219 (1.739-3.489) vs. 0.993 (0.719-1.577); P<0.001; Fig. 1D]. Moreover, AKIP1 mRNA expression was also able to differentiate between tumor tissue and adjacent tissue, with an AUC of 0.865 (95% CI, 0.797-0.932; Fig. 1E).

Association between tumor AKIP1 expression and clinical features in patients with endometrial carcinoma. Even though AKIP1 IHC score was not associated with myometrial invasion ≥1/2 (P=0.084; Fig. 2A) or cervical stroma invasion (P=0.141; Fig. 2B), increased AKIP1 IHC score was associated with lymphovascular invasion (P=0.007; Fig. 2C) and advanced FIGO stage (P=0.002; Fig. 2D) in the patients with endometrial carcinoma.

Although there was no association between AKIP1 mRNA expression and myometrial invasion ≥1/2 (P=0.238; Fig. 2E) or cervical invasion (P=0.297; Fig. 2F), elevated AKIP1 mRNA level was associated with lymphovascular invasion (P=0.020; Fig. 2G) and higher FIGO stage (P=0.027; Fig. 2H) in the patients with endometrial carcinoma.

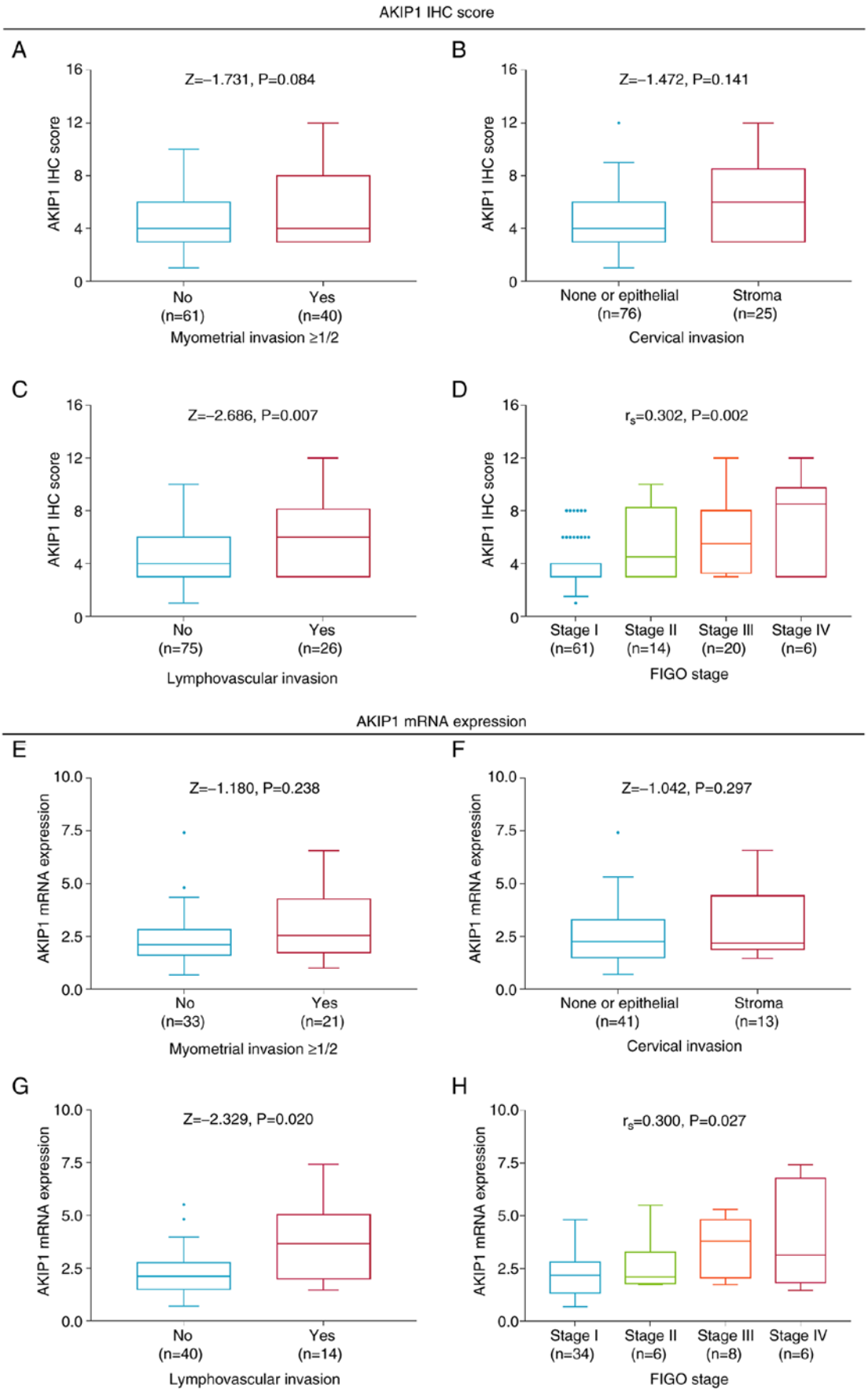

Figure 2. Increased AKIP1 expression is associated with advanced clinicopathological features. Association between AKIP1 IHC score and (A) myometrial invasion status, (B) cervical invasion status, (C) lymphovascular invasion status and (D) FIGO stage in patients with endometrial carcinoma. Association between AKIP1 mRNA expression and (E) myometrial invasion status, (F) cervical invasion status, (G) lymphovascular invasion status and (H) FIGO stage in patients with endometrial carcinoma. AKIP1, A-kinase-interacting protein 1; IHC, immunohistochemistry; FIGO, International Federation of Gynecology and Obstetrics.

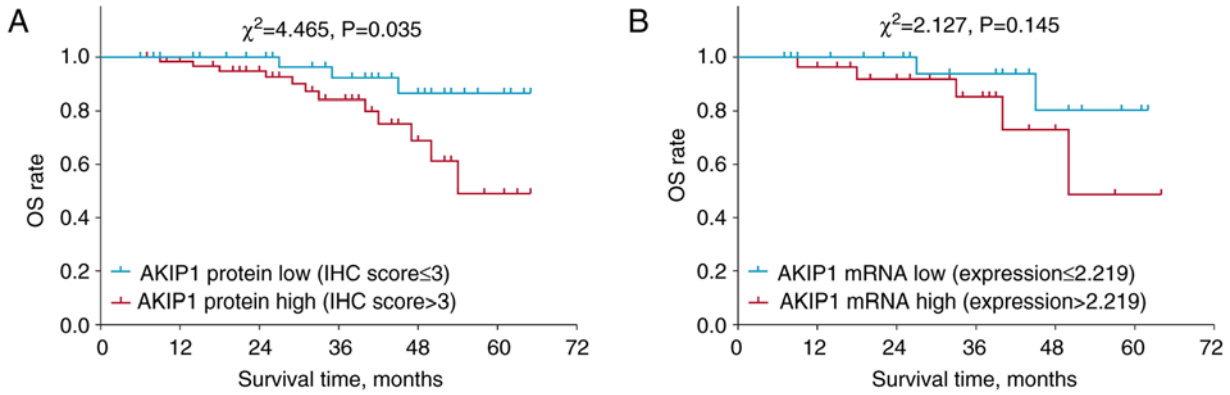

Figure 3. AKIP1 protein levels are associated with a shorter accumulating OS time. Association between (A) AKIP1 protein expression and (B) AKIP1 mRNA expression and accumulating OS in patients with endometrial carcinoma. AKIP1, A-kinase-interacting protein 1; IHC, immunohistochemistry; OS, overall survival.

Association between tumor AKIP1 expression and OS in patients with endometrial carcinoma. High AKIP1 protein expression (defined as an IHC score >3) was associated with a shorter accumulating OS time in the patients with endometrial carcinoma (P=0.035; Fig. 3A). However, high AKIP1 mRNA expression (defined as expression >2.219) was not associated with OS time (P=0.145; Fig. 3B). Further validation using The Cancer Genome Atlas database showed that high AKIP1 mRNA expression appeared to be associated with shorter OS time in the patients with endometrial carcinoma, although no statistical significance was found (P=0.052; Fig. S1).

Independent factors for OS in patients with endometrial carcinoma. Univariate Cox regression analysis identified that tumor AKIP1 protein expression (high vs. low) [hazard ratio (HR), 3.622; 95% confidence interval (CI), 1.013-12.946; P=0.048], cervical invasion (stromal vs. none or epithelial) (HR, 3.821; 95% CI, 1.382-10.565; P=0.010), lymphovascular invasion (yes vs. no) (HR, 3.769; 95% CI, 1.363-10.419; P=0.011) and higher FIGO stage (HR, 2.170; 95% CI, 1.382-3.406; P=0.001) were all associated with decreased OS time in the patients with endometrial carcinoma (Table II). Further multivariate Cox regression analysis found that tumor AKIP1 protein expression (high vs. low) (HR, 3.910; 95% CI, 1.095-13.965; P=0.036) and cervical invasion (stromal vs. none or epithelial) (HR, 4.104; 95% CI, 1.483-11.360; P=0.007) were independently associated with shorter OS time.

Effect of AKIP1 knockdown on chemosensitivity. Silencing of AKIP1 decreased the mRNA expression levels of AKIP1 in Ishikawa cells (P=0.002; Fig. S2), indicating successful transfection. Moreover, silencing of AKIP1 significantly decreased the relative cell viability of Ishikawa cells under treatment with 5 μM (P=0.015), 10 μM (P=0.045), 20 μM (P=0.018) and 40 μM (P=0.049) cisplatin compared with the NC siRNA group (Fig. 4A). However, silencing of AKIP1 only significantly decreased the relative cell viability of the Ishikawa cells under treatment with 4 nM paclitaxel (P=0.026), but not at other concentrations (all P>0.05) compared with the NC siRNA group (Fig. 4B). Furthermore, AKIP1 siRNA reduced the IC_{50} of paclitaxel and cisplatin (Fig. 4C and D).

Discussion

AKIP1 has been identified as a new and potential biomarker in several types of cancer in the clinical field (9-12). Previous studies have indicated that AKIP1 expression is upregulated in tumor tissue compared with that in non-cancerous adjacent tissue in patients with clear cell renal cell carcinoma, non-small cell lung cancer, colorectal cancer and hepatocellular carcinoma (9-12); however, to the best of our knowledge, there are no studies on the clinical value of AKIP1 in patients with endometrial carcinoma. Furthermore, most studies have detected AKIP1 protein expression by evaluating the IHC score from surgical specimens, while the mRNA expression levels of AKIP1 in these specimens have not been determined. Therefore, in the present study, tumor tissues and adjacent tissues were collected from patients with endometrial carcinoma in order to detect AKIP1 protein levels by IHC assay and AKIP1 mRNA expression levels by RT-qPCR, and to further explore the clinical value of AKIP1 in patients with endometrial carcinoma. An increase in AKIP1 expression was observed in the tumor tissues compared with that in the adjacent tissues, which other studies have suggested may be due to AKIP1 promoting angiogenesis through the regulation of the NF-κB and AKT pathways. This would lead to enhanced blood vessel formation, which is one of the hallmarks of endometrial carcinoma (8,21,22).

In terms of the association between AKIP1 expression and clinical features in patients with cancer, high AKIP1 expression has been reported to be associated with advanced TNM stage in clear cell renal carcinoma, non-small cell lung cancer and colorectal carcinoma (9-11). Moreover, high AKIP1 expression is also correlated with poor pathological differentiation and lymph node metastasis in patients with non-small cell lung cancer (11). In the present study, increased AKIP1 expression was associated with tumor invasion and advanced FIGO grade in the patients with endometrial carcinoma. The following reasons, suggested by other studies, could be applied to explain these findings: i) AKIP1 promotes EMT, which further enhances cancer invasion and metastasis by regulating the PI3K/Akt pathway, as well as mediating the expression of transcription factor zinc finger E-box binding homeobox 1 promoter and Slug, which thereby leads to the presence of tumor invasion in patients with endometrial

Table II. Univariate and multivariate Cox regression analyses on overall survival.

A, Univariate Cox regression analysis

Variables	P-value	HR	95% CI	
			Lower	Upper
Tumor AKIP1 protein (high vs. low)	0.048[a]	3.622	1.013	12.946
Age (≥60 vs. <60 years)	0.294	1.848	0.587	5.817
Menopausal status (post-menopause vs. pre-menopause)	0.652	1.408	0.317	6.248
Diabetes mellitus (yes vs. no)	0.473	1.482	0.505	4.348
Hypertension (yes vs. no)	0.343	0.609	0.219	1.696
Myometrial invasion ≥1/2 (yes vs. no)	0.277	1.757	0.636	4.855
Cervical invasion (stromal vs. none or epithelial)	0.010[a]	3.821	1.382	10.565
Lymphovascular invasion (yes vs. no)	0.011[a]	3.769	1.363	10.419
FIGO stage (III/IV vs. I/II)	0.001[a]	2.170	1.382	3.406

B, Multivariate Cox regression analysis

Variables	P-value	HR	95% CI	
			Lower	Upper
Tumor AKIP1 protein (high vs. low)	0.036[a]	3.910	1.095	13.965
Cervical invasion (stromal vs. none or epithelial)	0.007[a]	4.104	1.483	11.360

[a]P<0.05. HR, hazard ratio; CI, confidence interval; AKIP1, A-kinase-interacting protein 1; FIGO, International Federation of Gynecology and Obstetrics.

carcinoma (7,23,24). ii) Occurrence of lymphovascular invasion, myometrial invasion ≥1/2 and cervical stroma invasion are associated with advanced FIGO grade in patients with endometrial carcinoma (25,26). Therefore, elevated AKIP1 expression is associated with advanced FIGO stage in patients with endometrial carcinoma.

The association between AKIP1 and prognosis in patients with cancer is also noteworthy. For example, high AKIP1 protein expression has been shown to be associated with early recurrence (defined as recurrence within 2 years) and is independently correlated with shorter recurrence-free survival times in patients with hepatocellular carcinoma (12). Moreover, high AKIP1 expression is an independent factor for shorter OS time in patients with clear cell renal carcinoma, non-small cell lung cancer and colorectal carcinoma (9-11). In the present study, high tumor AKIP1 protein expression was independently associated with decreased OS time in the patients with endometrial carcinoma. Possible reasons to explain these findings, as suggested by previous studies, were: i) Elevated tumor AKIP1 expression promoting endometrial carcinoma cell invasion and migration through the mediation of multiple pathways (such as the Akt/GSK-3β and PI3K/Akt pathways), thus leading to an enhanced risk of tumor recurrence and an unfavorable prognosis in patients with endometrial carcinoma (7,27,28). ii) As illustrated in the present *in vitro* study, silencing of AKIP1 enhanced the chemosensitivity to cisplatin and paclitaxel in an endometrial carcinoma cell line, thus its overexpression may decrease the postoperative chemosensitivity, thereby leading to an unfavorable prognosis in patients with endometrial carcinoma.

Resistance to chemotherapy drugs is another factor contributing to an unfavorable prognosis in patients with endometrial carcinoma (29). A previous study indicated that overexpression of AKIP1 promoted the resistance to temozolomide by regulating CXCL1- and CXCL8-mediated NF-κB and AKT pathways in glioblastoma cells (30). Moreover, AKIP1 was shown to be associated with chemotherapy resistance in ovarian cancer using transcriptomic data (31). However, to the best of our knowledge, no relevant study has investigated the effect of AKIP1 on chemosensitivity in endometrial carcinoma. In the present study, it was shown that silencing of AKIP1 could enhance the chemosensitivity to cisplatin and paclitaxel (to some extent) in endometrial carcinoma cells, which may be explained by the silencing of AKIP1 regulating multiple chemosensitivity-related signaling pathways (such as NF-κB and AKT pathways) to enhance cisplatin and paclitaxel chemosensitivity in endometrial carcinoma (30,32,33). The present findings suggested that AKIP1 was involved in regulating the chemosensitivity of the endometrial carcinoma cell line, which provided some evidence for further therapeutic intervention.

The present study has several limitations. For instance, the sample size was relatively small; therefore, further studies with a larger sample size are needed to validate the clinical value of AKIP1 in patients with endometrial carcinoma.

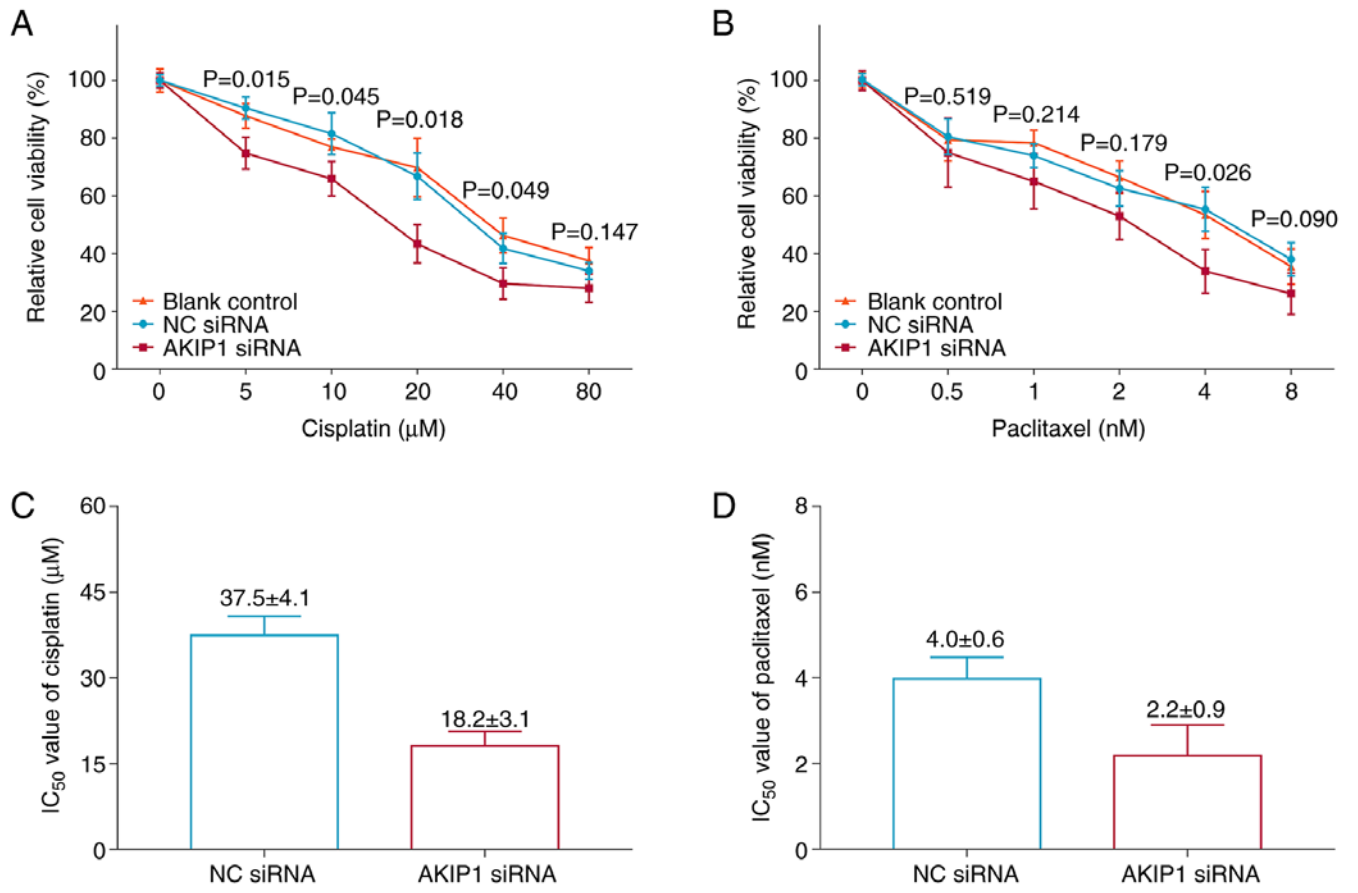

Figure 4. Silencing of AKIP1 enhances Ishikawa cell chemosensitivity. Effect of AKIP1 siRNA on relative cell viability under different concentrations of (A) cisplatin and (B) paclitaxel treatment in Ishikawa cells (AKIP1 siRNA group vs. NC siRNA group). Comparison of IC_{50} value of (C) cisplatin and (D) paclitaxel in Ishikawa cells transfected with NC siRNA and AKIP siRNA (data are presented as mean ± SD). AKIP1, A-kinase-interacting protein 1; NC, negative control; siRNA, small interfering RNA; IC_{50}, half maximal inhibitory concentration; SD, standard deviation.

Furthermore, most patients in the current study did not live in the local region and they did not receive long-term routine management/follow-up after surgery in the same hospital; therefore, the present study did not evaluate the disease relapse information of the patients. Furthermore, the present study did not detect the detailed molecular mechanism of AKIP1 with regard to its regulation of chemosensitivity in endometrial carcinoma, which could be explored in future studies.

In conclusion, the present study indicated that elevated AKIP1 expression was associated with tumor invasion, shorter survival times and reduced chemosensitivity in patients with endometrial carcinoma.

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Availability of data and materials

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

Authors' contributions

FZ conceived and designed the study. ALL, TYZ, ZLM and YLX were involved in performing the experiments and collected the data. ALL, AJL, XPG and FZ analyzed the data. XPG prepared the figures and tables. ALL and AJL confirm the authenticity of all the raw data. ALL, AJL, TYZ, ZLM and YLX wrote the manuscript. XPG and FZ revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Approval was acquired from the Institutional Review Board (IRB) of Handan Central Hospital before the implementation of the study (approval number HDZXYY-Ethics-2021015). The requirement for written informed consent was waived by the IRB.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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