IGFBP-rP1 affects the proliferation, apoptosis and macrophage polarization of endometrial cancer cells by regulating the PI3K/AKT pathway

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Abstract. Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) is a potential tumor suppressor gene in a variety of cancers including colorectal cancer and breast cancer. However, its role and the potential mechanism in endometrial carcinoma (EC) are still unclear. The purpose of this study was to explore the effect of IGFBP-rP1 on EC cell proliferation and apoptosis and its underlying mechanism. Western blot analysis and reverse transcription-quantitative PCR were used to assess protein and gene expression levels of IGFBP-rP1 in EC cells. Overexpression of IGFBP-rP1 and/or AKT serine/threonine kinase was used to analyze their effects on cell proliferation and apoptosis of the EC cells. Co-immunoprecipitation and glutathione S transferase pull-down assays were used to analyze the interaction between IGFBP-rP1 and AKT. The expression of IGFBP-rP1 in EC cells was downregulated. Overexpression of IGFBP-rP1 inhibited the proliferation and induced apoptosis of EC cells, which were abolished by the overexpression of AKT. In addition, IGFBP-rP1 directly interacted with AKT to inhibit PI3K/AKT signaling. Additionally, M0 macrophages were induced by EC cells to differentiate into M2 macrophages, which was reversed by IGFBP-rP1. Overexpression of AKT in EC cells abolished the inhibitory effect of IGFBP-rP1 on M2 polarization. IGFBP-rP1 as an oncogenic factor inhibits M2 polarization of TAMs through PI3K/AKT signaling pathway and may be a potentially valuable target for EC therapy.

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Key words: endometrial cancer, insulin-like growth factor binding protein-related protein 1, M2 macrophages, tumor microenvironment

Introduction

Endometrial cancer (EC) is one of the commonest gynecological malignancies and has the sixth-highest incidence of cancer in women worldwide (1). Despite significant advances in the diagnosis and treatment, the prognosis for patients with advanced and recurring EC remains poor (2). Exploring the molecular mechanisms of EC to develop new treatment strategies is critical for improving the prognosis of patients.

The tumor microenvironment (TME) is linked to the tumorigenesis and progression of the disease (3). The TME consists primarily of cancer cells, cancer-associated fibroblasts, immune cells and non-cellular components, with macrophages being the most abundant immune cells (4). M2 macrophages, also known as tumor-associated macrophages (TAMs), are closely linked to the development and progression of various cancers (5,6). TAMs secrete a variety of mediators, such as cytokines and chemokines, to suppress anti-tumor immune responses and promote cancer cell proliferation, invasion and spreading (7,8). Studies show that tumor cells direct macrophages toward the M2 phenotype to promote malignant progression (9). Inhibiting M2 macrophage polarization can slow the progression of cancer (10). The underlying mechanisms by which cancer cells control macrophage M2 polarization, however, remain to be elucidated.

Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1, also known as IGFBP7) is a secreted protein of the insulin-like growth factor binding protein superfamily that has been linked to insulin resistance (11). IGFBP-rP1 appears to be a tumor suppressor in a variety of cancers, including colorectal cancer and breast cancer (11,12). A previous study has linked elevated IGFBP-rP1 levels to a lower risk of EC (13). Hu *et al* (14) proposed that IGFBP-rP1 plays an active role in promoting the interaction between cancer cells and TME, which could explain why cancer cells adhere, invade and migrate. The potential mechanism of IGFBP-rP1 in EC, however, remains unknown.

The present study aimed to look at the effect of IGFBP-rP1 on EC cell proliferation and apoptosis, as well at the role of IGFBP-rP1 in the formation of M2 TAMs.

Materials and methods

Cell cultures. Human endometrial cancer cell lines (Ishikawa, HEC-1A, RL95-2, HEC-1B and AN3CA) and human mononuclear cells (THP-1) were purchased from Procell Life Science & Technology Co., Ltd. All cells were cultured in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 μg/ml penicillin and 100 μg/ml streptomycin (HyClone; Cytiva) and cultured at 37˚C in 5% CO_2.

Reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from EC cells ($1x10^5$ cells/well) according to the manufacturer's protocols using the TRIzol® kit (Thermo Fisher Scientific, Inc.). A cDNA synthesis kit (Thermo Fisher Scientific, Inc.) was used to transcribe total RNA into cDNA in accordance with the manufacturer's protocols. The SYBR Green One-step RT-PCR Master Mix (Thermo Fisher Scientific, Inc.) and 7900 real-time PCR system (Thermo Fisher Scientific, Inc.) was used for RT-qPCR. The following thermocycling conditions were used for qPCR: Initial pre-denaturation at 95˚C for 120 sec; 40 cycles of denaturation at 95˚C for 15 sec, annealing at 58˚C for 30 sec and elongation at 72˚C for 30 sec. The primers used were: IGFBP-rP1, Forward 5'-AGC TGT GAG GTC ATC GGA AT-3', Reverse 5'-CAG CAC CCA GCC AGT TAC TT-3'; GAPDH, Forward 5'-GGA GCG AGA TCC CTC CAA AAT-3', Reverse 5'-GGC TGT TGT CAT ACT TCT CAT GG-3'. The $2^{-\Delta\Delta Cq}$ method was used to calculate fold changes in the gene expression normalized to GAPDH (15). At least three replicate wells were performed for each group.

Western blot analysis. RIPA lysis buffer (Beyotime Institute of Biotechnology) was used to extract total protein from EC cells. The protein concentration of the supernatants was assessed using the Bradford Protein Assay kit (Beyotime Institute of Biotechnology). Equal amounts of total protein (30 μg) was then separated on 10% SDS-PAGE before being transferred to PVDF membranes. TBS-Tween-20 (Beyotime Institute of Biotechnology) containing 5% skimmed milk powder was used to block the membranes for 1 h at 37˚C, which were then incubated with primary antibodies overnight at 4˚C. The primary antibodies were as follows: anti-IGFBP-rP1 (cat. no. MAB1334; 1:500; R&D Systems), anti-AKT (cat. no. ab108202; 1:500; Abcam), anti-phosphorylated (p-)AKT (cat. no. 4060; 1:2,000; Cell Signaling Technology, Inc.), anti-Bax (cat. no. ab32503; 1:1,000; Abcam), anti-Caspase 3 (cat no. 19677-1-AP; 1:500; ProteinTech Group, Inc.), anti-Bcl-2 (cat. no. ab182858; 1:2,000; Abcam) and anti-GAPDH (cat. no. ab8245; 1:1,000; Abcam). The membranes were incubated with HRP-conjugated anti-mouse IgG (cat. no. 7076; 1:5,000; Cell Signaling Technology, Inc.) secondary antibody for 2 h at 37˚C. The membranes were developed using an enhanced chemiluminescence system (Beyotime Institute of Biotechnology). The density of each protein blot was compared with that of GAPDH using ImageJ software (version 1.46r; National Institutes of Health) and was shown as a ratio to the endogenous control.

Transfections. The pcDNA3.1-IGFBP-rP1 (p-IGFBP7) containing full-length IGFBP-rP1 coding sequence (GeneBank accession, BC017201.2) and empty pcDNA3.1 (p-NC1), pcDNA3.1-AKT (p-AKT) containing full-length AKT coding sequence (GeneBank accession, MG516906.1) and empty pcDNA3.1 (p-NC2) were obtained from Sangon Biotech Co., Ltd. HEC-1B and AN3CA cells were seeded into six-well plates and cultured for 24 h until 80% confluence. Then, HEC-1B and AN3CA cells were transfected with p-IGFBP7 (2 μg) or p-AKT (2 μg) using Lipofectamine® 3000 Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's instructions for 6 h at 37˚C. In rescue experiments, cells were co-transfected with p-IGFBP7 (1 μg) and p-AKT (1 μg) at the same time. At 48 h post-transfection, cells were collected and used for subsequent experiments.

MTT assay. The cell proliferation was measured using the MTT assay (MilliporeSigma). Briefly, HEC-1B and AN3CA cells were inoculated in 96-well plates containing 200 μl of DMEM medium at $5x10^3$ cells/well. In order to determine the adherence of cells to the floor of plate wells, the plates were incubated for 24 h at 37˚C and 5% carbon dioxide. After the indicated treatment, cells were incubated with 5 mg/ml MTT solution (20 μl for each well) and the plates were incubated at 37˚C for 4 h. The wells were then emptied and 150 μl of dimethyl sulfoxide (DMSO) was added to each well. A Multiska FC microplate reader (Thermo Fisher Scientific, Inc.) was then used to calculate the optical density at 490 nm.

Flow cytometry. Apoptosis was evaluated using Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) (Beyotime Institute of Biotechnology). Briefly, after the indicated treatment, the cells were collected and washed with PBS. Annexin-binding buffer was used to re-suspend HEC-1B and AN3CA cells that were then incubated with Annexin V-FITC and PI for 10 min at room temperature. Apoptosis was assessed by flow cytometer (Beckman Coulter, Inc.) with Cell Quest software v6.0 (BD Biosciences). FITC/PI denoted living cells, FITC+/PI indicated early apoptotic cells, FITC+/PI+ represented late apoptotic cells and FITC/PI+ depicted necrotic cells.

Glutathione S-transferase (GST) pull-down assay. GST or GST-fusion proteins were expressed and purified in accordance with the manufacturer's protocol (Cytiva). His-tagged Protein Purification kit was purchased from GenScript. HEC-1B and AN3CA cells were transformed with the plasmid expressing GST fusion proteins (2 μg) or GST control (2 μg). *In vitro*-translated His-IGFBP-rP1 (2 μg) was incubated with GST-AKT fusion proteins (2 μg) or GST alone (2 μg) for 3 h at 4˚C. GST or GST-AKT were bound to glutathione-Sepharose beads (Cytiva). The mixture was washed eight times with PBS to thoroughly remove unbound proteins. A volume of 50 μl of SDS-PAGE loading buffer (1x) was added, boiled for 5 min and centrifuged at 12,000 x g for 1 min at 4˚C. The absorbed proteins were analyzed using western blotting.

Co-immunoprecipitation (co-IP) assay. Whole-cell lysate (400 μg) were treated with anti-AKT antibodies (1:100; cat. no. ab183556; Abcam) or IgG control antibody (1:100;

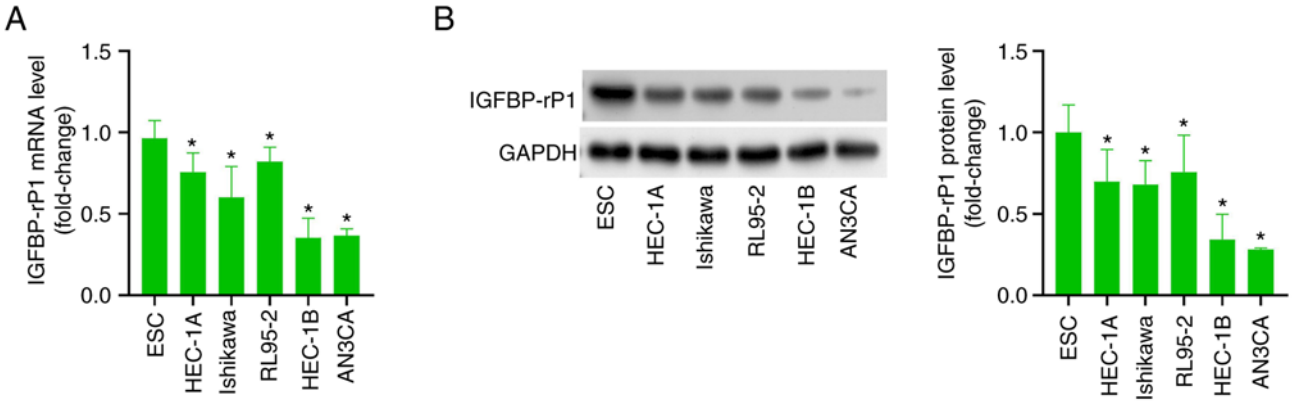

Figure 1. The expression of IGFBP-rP1 in EC cells is downregulated. (A) The level of IGFBP-rP1 mRNA in EC cells was analyzed using reverse transcription-quantitative PCR. (B) The IGFBP-rP1 protein level in EC cells was analyzed using western blotting. *P<0.05. IGFBP-rP1, insulin-like growth factor binding protein-related protein 1; EC, endometrial carcinoma; ESC, endometrial epithelial cells.

cat. no. ab172730; Abcam) in TBS buffer (40 mM Tris-HCl pH 7.5, 130 mM NaCl) for 1 h at room temperature, and then was centrifuged at 14,000 x g for 15 min at 4˚C. Next, protein A agarose beads (2 μg/ml) were added to the supernatant; then, it was shaken at 4˚C for 10 min on horizontal ice to remove non-specific foreign proteins and reduce the background. Then, protein A beads (2 μg/ml; cat. no. #sc-2003; Santa Cruz Biotechnology, Inc.) were removed after centrifuging at 14,000 x g for 15 min at 4˚C. The mixture of antibody and tissue lysate was slowly shaken at 4˚C overnight. Subsequently, 100 μl of protein A agarose beads were added to capture the antibody and its bound proteins, and the antigen-antibody mixture was slowly shaken at 4˚C overnight. The mixture was centrifuged at 14,000 x g for 5 sec at 4˚C, the agarose bead antibody complex was collected, the supernatant was removed and washed with cooled PBS buffer for three times. Then, the released proteins were analyzed by western blot analysis using anti-IGFBP-rP1 (1:500) or anti-AKT antibodies (1:500).

Production and differentiation of macrophages. THP-1 cells were treated with 100 ng/ml Phorbol 12-myristate 13-acetate (PMA) (cat. no. P1585; MilliporeSigma) for 24 h at room temperature to generate THP-1 macrophages (M0 macrophages) (16). To simulate the formation of TAMs, M0 macrophages were co-cultured with HEC-1B and AN3CA cells transfected with IGFBP-rP1 overexpression vector (p-IGFBP7) and/or AKT overexpression vector (p-AKT) in a 6-well Transwell co-culture system for 48 h at room temperature.

ELISA. The concentration of inducible nitric oxide synthase (iNOS; cat. no. ab253217; Abcam), TNF-α (cat. no. ab181421; Abcam), CDl63 (cat. no. ab274394; Abcam), arginase-1 (Arg-1; cat. no. BMS2216; Thermo Fisher Scientific, Inc.) and mannose receptor (MR; cat. no. ab277420; Abcam) in the culture medium was measured by ELISA-kits according to the manufacturer's instructions, respectively.

Statistical analysis. All statistical analyses were performed using SPSS 19.0 statistical software (IBM Corp.). Data were analyzed by unpaired Student's t-test or one-way ANOVA followed by Tukey's post hoc test and were presented as the mean ± standard deviation of three independent experiments. P<0.05 was considered to indicate a statistically significant difference.

Results

The expression of IGFBP-rP1 in EC cells is downregulated. RT-qPCR showed that compared with normal endometrial epithelial (ESC) cells, IGFBP-rP1 mRNA level in Ishikawa, HEC-1A and RL95-2 was markedly diminished, especially in HEC-1B and AN3CA cells (Fig. 1A). Similarly, the expression of IGFBP-rP1 protein in EC cell lines was significantly lower than that of normal ESC cells, especially in HEC-1B and AN3CA cells (Fig. 1B). Therefore, HEC-1B and AN3CA cells were chosen for follow-up experiments.

Overexpression of IGFBP-rP1 affects the proliferation and apoptosis of EC cells by regulating the PI3K/AKT pathway. To overexpress IGFBP-rP1, the pcDNA3.1-IGFBP-rP1 (p-IGFBP7) vector was transfected into HEC-1B and AN3CA cells. Compared with the p-NC group, IGFBP-rP1 mRNA and protein levels in the p-IGFBP7 group were increased significantly, confirming the successful transfection (Fig. 2A and B). Successful transfection of pcDNA3.1-AKT vector significantly increased the expression of AKT mRNA and protein in HEC-1B and AN3CA cells (Fig. 2A and B). Compared with the p-NC group, the expression of AKT and p-AKT in HEC-1B and AN3CA cells in the p-IGFBP7 group was significantly reduced (Fig. 2C and D), which suggested that overexpression of IGFBP7 inhibited the activation of PI3K/AKT pathway in HEC-1B and AN3CA cells. To verify this conclusion, we co-transfected HEC-1B and AN3CA cells with the p-IGFBP7 and p-AKT. Compared to the p-IGFBP7 group, the expression of AKT and p-AKT proteins were markedly increased in the p-IGFBP7 + p-AKT group (Fig. 2C and D).

To further evaluate the effect of IGFBP-rP1 on the biological characteristics of EC cells, MTT assay was used to evaluate cell proliferation. The results showed that compared to the p-NC group, the proliferation of HEC-1B and AN3CA cells were markedly decreased in p-IGFBP7 group (Fig. 2E). Additionally, the proliferation of EC cells was significantly

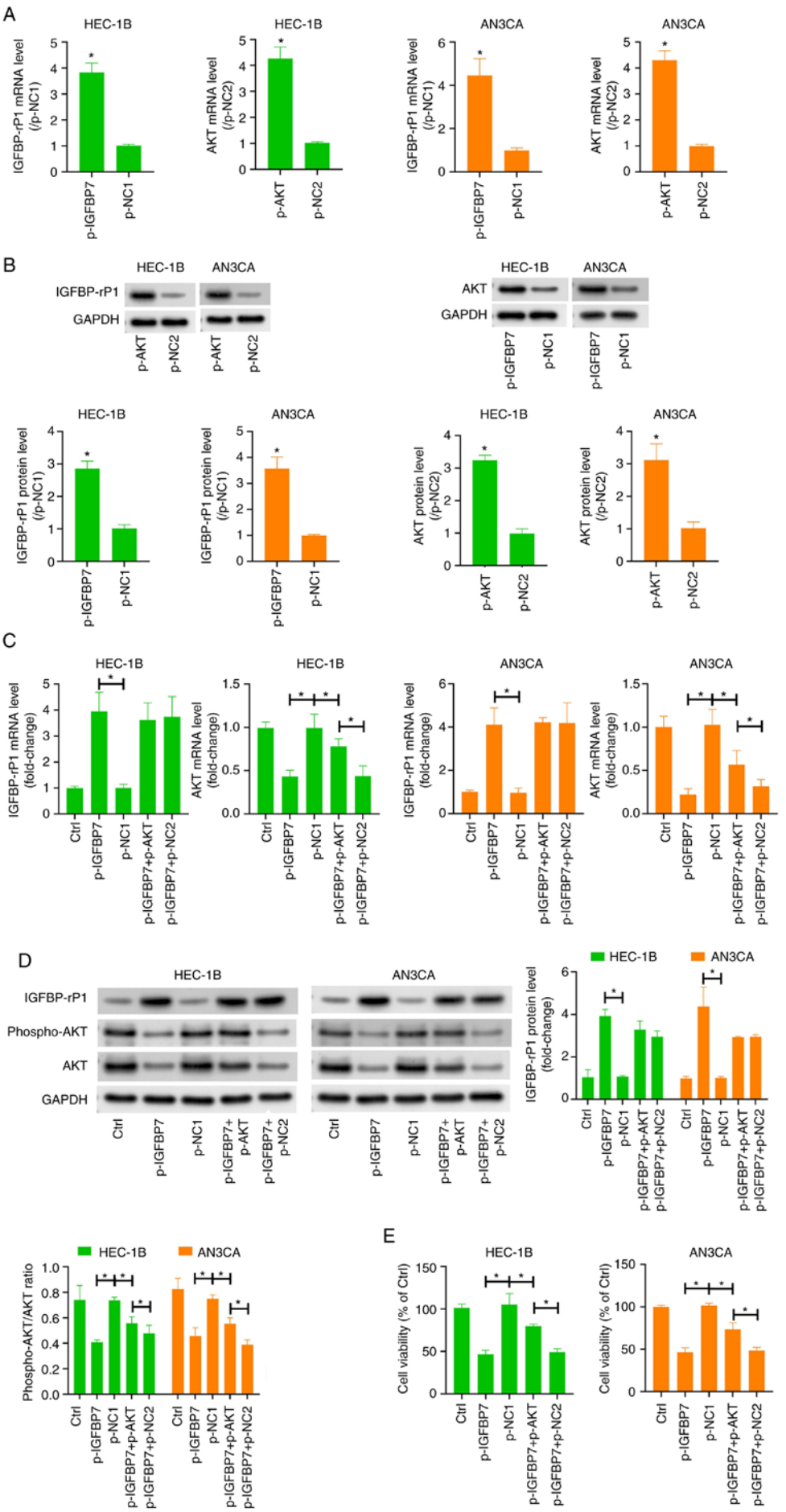

Figure 2. Continued.

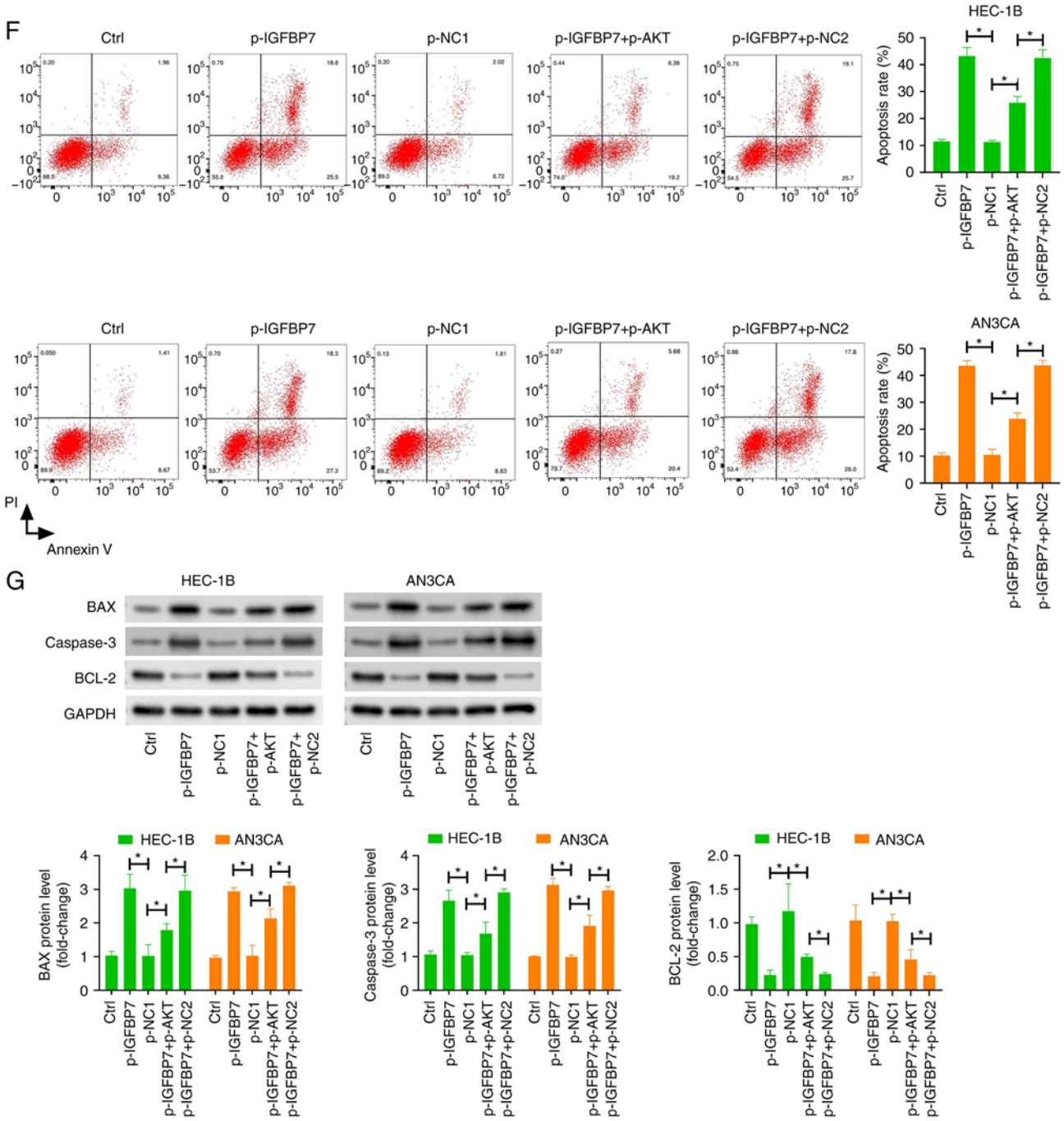

Figure 2. Overexpression of IGFBP-rP1 affects the proliferation and apoptosis of EC cells by regulating the PI3K/AKT pathway. (A) The levels of IGFBP-rP1 and AKT mRNA in HEC-1B and AN3CA cells transfected with p-IGFBP7 or p-AKT were analyzed using RT-qPCR. (B) The protein level of IGFBP-rP1 and AKT in HEC-1B and AN3CA cells transfected with p-IGFBP7 or p-AKT were assessed. (C) The IGFBP-rP1 and AKT mRNA in HEC-1B and AN3CA cells transfected with p-IGFBP7 and p-AKT were analyzed using RT-qPCR. (D) The expression of IGFBP-rP1, p-AKT and AKT in HEC-1B and AN3CA cells transfected with p-IGFBP7 and p-AKT were analyzed using RT-qPCR. (E) MTT assay was used to assess the proliferation of HEC-1B and AN3CA cells transfected with p-IGFBP7 and p-AKT. (F) Flow cytometry was used to assess apoptosis of HEC-1B and AN3CA cells transfected with p-IGFBP7 and p-AKT. (G) The expression of apoptosis-related proteins BAX, Caspase-3 and BCL-2 were analyzed using western blotting. *P<0.05. IGFBP-rP1, insulin-like growth factor binding protein-related protein 1; EC, endometrial carcinoma; p-, phosphorylated; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control.

increased in p-IGFBP7 + p-AKT group compared with the p-IGFBP7 + p-NC2 group (Fig. 2E). Flow cytometry also showed that the apoptosis of HEC-1B and AN3CA cells was evidently increased in p-IGFBP7 group compared with the p-NC1 group (Fig. 2F). Consistently, the expression of BAX and Caspase-3, while the expression of BCL-2 was markedly decreased in p-IGFBP7 group compared with the p-NC1 group (Fig. 2G), which further confirmed the induction of EC cell apoptosis by IGFBP-rP1. Compared to the p-IGFBP7 group, the apoptosis of HEC-1B and AN3CA cells was markedly decreased in p-IGFBP7 + p-AKT group (Fig. 2F and G). Taken together, our results indicate that IGFBP-rP1 inhibited the malignant phenotype of EC cells by inhibiting the activated PI3K/AKT pathway.

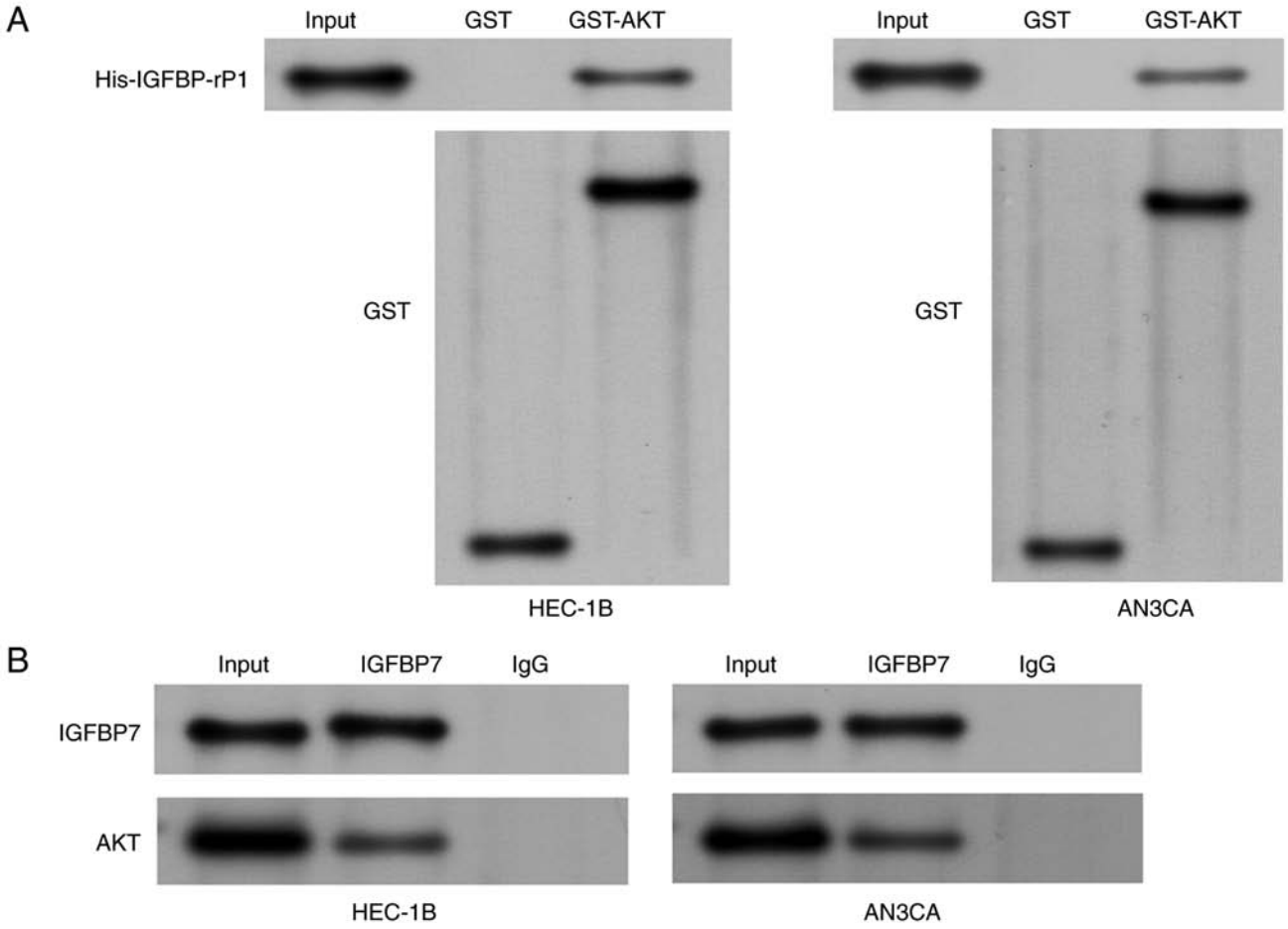

Figure 3. The interaction of IGFBP-rP1 and AKT in EC cells. (A) GST and GST-tagged AKT were purified with glutathione agarose beads and incubated with His-IGFBP-rP1. (B) HEC-1B and AN3CA cells were lysed and incubated with anti-IGFBP-rP1 antibody followed by western blot assay with anti-AKT antibody and IgG as negative control. GST, glutathione S-transferase; IGFBP-rP1, insulin-like growth factor binding protein-related protein 1.

The interaction of IGFBP-rP1 and AKT in EC cells. The association between IGFBP-rP1 and AKT was evaluated. A GST pull-down assay was performed to assess the binding ability of IGFBP-rP1 to AKT (Fig. 3A). The results confirmed the direct interaction between IGFBP-rP1 and AKT. The direct interaction of IGFBP-rP1 and AKT was also confirmed by co-immunoprecipitation assay in HEC-1B and AN3CA cells (Fig. 3B).

Overexpression of IGFBP-rP1 affects the polarization of EC-related macrophages by regulating the PI3K/AKT pathway. TAMs are a type of M2 macrophages with tumor-promoting effects that play a key role in mediating the connection between cells in the tumor microenvironment (5). In order to confirm the effect of IGFBP-rP1 on macrophages at the cellular level, human THP-1 monocytes were treated with PMA for 24 h to induce M0 macrophages (15). Subsequently, M0 cells were co-cultured with EC cells overexpressing IGFBP-rP1 and/or overexpressing AKT for 48 h to produce TAMs. Compared with M0, TAMs showed lower levels of M1 markers iNOS (Fig. 4A) and TNF-α (Fig. 4B) and higher levels of M2 markers CD163 (Fig. 4C), Arg-1 (Fig. 4D) and MR (Fig. 4E), suggesting that M0 macrophages were induced by cancer cells to differentiate into M2 macrophages. Compared with M0 + p-NC1 group, the expression of iNOS and TNF-α was increased (Fig. 4A and B), while the expression of CD163, Arg-1 and MR was decreased in M0 + p-IGFBP7 group (Fig. 4C-E), suggesting that overexpression of IGFBP-rP1 inhibited M2 polarization. However, compared to the M0 + p-IGFBP7 + p-NC2 group, the expression of iNOS and TNF-α was decreased, while the expression of CD163, Arg-1 and MR was increased in M0 + p-IGFBP7 + p-AKT group, suggesting that the overexpression of AKT in EC cells abolished the inhibitory effect of IGFBP-rP1 on M2 polarization (Fig. 4A-D).

Discussion

IGFBP-rP1 is a potential tumor suppressor gene in a variety of cancers, including EC (13). Nonetheless, its mechanism in EC remains to be elucidated. The present study showed that IGFBP-rP1 has low expression levels in EC cells. It was found in *in vitro* experiments that overexpressed IGFBP-rP1 inhibited the proliferation and induced apoptosis of EC cells. These findings suggested that IGFBP-rP1 might play a tumor suppressor role in EC.

The PI3K/AKT pathway is a classic pathway that regulates cell proliferation, apoptosis and metastasis (16,17). A previous study reported that the activation of the PI3K/AKT pathway is related to the continuous growth of various solid tumors,

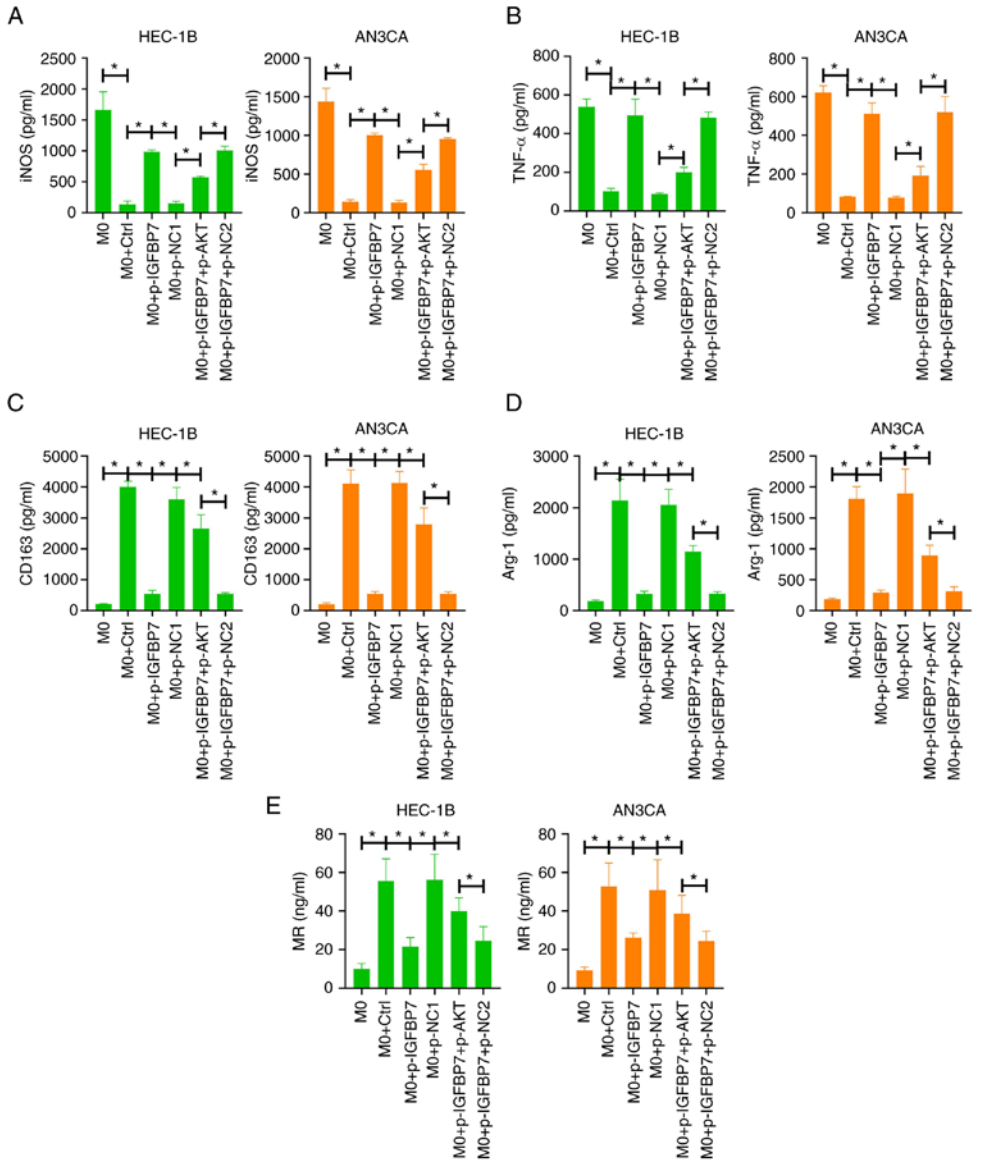

Figure 4. Overexpression of IGFBP-rP1 affects the polarization of EC-related macrophages by regulating the PI3K/AKT pathway. Human THP-1 monocytes were treated with PMA for 24 h to induce M0 macrophages. Then, M0 cells were co-cultured with EC cells overexpressing IGFBP-rP1 and/or overexpressing AKT for 48 h to produce TAMs. The level of M1 markers, (A) iNOS and (B) TNF-α and M2 markers, (C) CD163, (D) Arg-1 and (E) MR were analyzed using ELISA. *P<0.05. IGFBP-rP1, insulin-like growth factor binding protein-related protein 1; EC, endometrial carcinoma; TAMs, tumor-associated macrophages; iNOS, inducible nitric oxide synthase; NC, negative control; Arg-1, arginase-1; MR, mannose receptor.

including EC (18). AKT plays a major role in this signal pathway. p-AKT is related to the disorder of apoptosis, proliferation and cell motility because of its role in inducing signals that interfere with the normal regulatory mechanisms that activate the mTOR (19). In the current study, it was observed that overexpression of IGFBP-rP1 reduced levels of p-AKT in EC cells, indicating that the activation of the PI3K/AKT signaling pathway was repressed. Additionally, the overexpression of AKT effectively reversed the reduced proliferation and increased apoptosis caused by IGFBP-rP1. These findings suggested that IGFBP-rP1 inhibited the PI3K/AKT signaling pathway to exert a tumor suppressor effect in EC. Importantly, through co-IP and GST pull-down assays, it was also confirmed that AKT is a key protein interacting with IGFBP-rP1. Based on these data, it was hypothesized that IGFBP-rP1 directly binds to AKT to block the phosphorylation of AKT, thereby inhibiting the PI3K/AKT pathway.

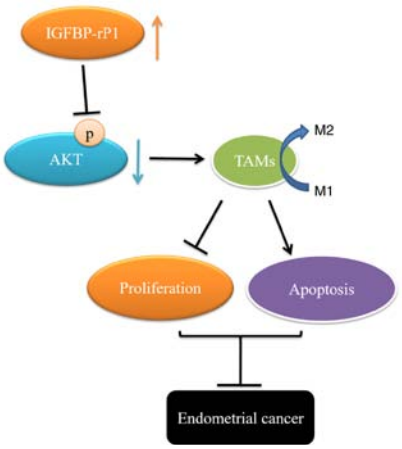

Figure 5. The schematic of the regulation of the P13K/AKT pathway by IGFBP-1rP1. IGFBP-rP1, insulin-like growth factor binding protein-related protein 1, p, phosphorylation; TAMs, tumor-associated macrophages.

Previous studies provide novel understanding into the appearance of TAMs in the tumor microenvironment (20,21). Studies indicate that the existence of TAMs at tumor sites is closely related to tumor progression (22,23). Cassetta *et al* (24) isolated TAMs from breast cancer and EC tissues and found that the TAMs population positively correlated with poorer clinical prognosis. TAMs, therefore, may be crucial to the occurrence and development of EC. Gu *et al* (25) proposed that blocking M2 macrophages in TME may be a promising target for EC tumor immunotherapy. The present study used macrophages as a study subject to observe the effect of IGFBP-Rp1 on macrophage polarization by co-culturing macrophages with EC cells to mimic the inflammatory microenvironment of EC *in vitro*. As expected, M2 macrophage markers were significantly upregulated after incubating M0 macrophages with the EC cells. In addition, by co-culturing M0 macrophages with the EC cells transfected with IGFBP-rP1 overexpression vector, the changes in cytokine markers in the macrophages were blocked. These data suggested that the high expression of IGFBP-rP1 inhibited the M2 differentiation induced by the EC cells. The role of PI3K/AKT pathway in TAMs has also been studied. The uncontrolled activation of the PI3K/AKT pathway induces immune tolerance TME and regulates the transition between immune stimulation and immunosuppression of TAMs (26). Inhibition of PI3Kγ has been proposed as a macrophage-based cancer treatment strategy (27). Additionally, changes in AKT isoforms or AKT activity levels in macrophages determine the viability of monocytes/macrophages (28,29). The present study showed that overexpression of AKT in EC cells abolished the effect of overexpression of IGFBP-rP1 on M2 polarization, indicating that this regulation was dependent on the PI3K/AKT pathway. IGFBP-rP1, therefore, acted as an inhibitor of the M2 polarization of TAMs through the PI3K/AKT signaling pathway (Fig. 5).

In conclusion, the present study confirmed that IGFBP-rP1 promotes the proliferation and induces apoptosis of EC cells. Additionally, IGFBP-rP1 directly binds to AKT to block the phosphorylation of AKT, thereby inhibiting the PI3K/AKT pathway. The present study also proved that IGFBP-rP1 recruited M2 TAMs through PI3K/AKT signals. These results indicated that IGFBP-rP1 can be a capability marker for the treatment of EC in the future. The lack of clinical and animal studies is a limitation of the present study and will be fully researched in future studies.

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Availability of data and materials

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

Authors' contributions

JG was the main contributor for designing the study and writing the manuscript. SS, JL and CW contributed to conducting the experiments. RD, YH and CZ analyzed the data and revised the manuscript. All authors read and approved the final manuscript. RD and CZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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