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The role of insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) in endometrial carcinoma.

Haixia Qin[1,2], Danxia Chu[2], Lijun Fan[2], Yanmin Li[2], Ya Xie[2], Ruixia Guo[2*]

[1]Department of Obstetrics and Gynecology, the First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan, PR China

[2]Department of Obstetrics and Gynecology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, PR China

Abstract

Objective: Insulin resistance is a high risk factor of Endometrial Carcinoma (EC). Insulin like Growth Factor Binding Protein-Related Protein 1 (IGFBP-rP1) could lead to insulin resistance due to its high affinity with insulin. The role of IGFBP-rP1 in EC is still unclear.

Method: The IGFBP-rP1 was overexpressed at the cellular level first, and then its effect on the growth of endometrial carcinoma cells was observed and its regulatory mechanism was investigated.

Results: Our study showed that IGFBP-rP1 upregulation decreased the proliferation of cells and the proportion of S+G_2/M phase, but increased the apoptosis of human endometrial cancer cells HEC-1A. The expression of p-ERK1/2 was inhibited, while the concentration of rhIGFBP-rP1 increased. Nevertheless, IGFBP-rP1 was highly expressed in EC tissues. Further examination revealed that the DNA hypomethylation in the promoter region of IGFBP-rP1 was correlated with the high mRNA and protein expression levels of IGFBP-rP1 in EC tissues.

Conclusion: These results indicate that the DNA hypomethylation of IGFBP-rP1 can be used to predict high risk of EC. Further study may be required to confirm the predictor role of the methylation of IGFBP-rP1 in EC.

Keywords: Endometrial carcinoma, IGFBP-rP1, Methylation, ERK1/2.

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Introduction

Endometrial Carcinoma (EC) is one of the three most malignant tumors in female reproductive duct, accounting for 7% of female malignant tumor. According to the National Cancer Institute (NCI), the incidence of EC is becoming the highest in the malignant tumors of female reproductive duct [1]. Epidemiological studies have shown that metabolic syndrome, which is manifested as obesity, hypertension and diabetes, is a high risk factor to EC [2]. These high risk factors have a common pathophysiological basis, i.e. insulin resistance and the following hyperinsulinemia [3].

IGFBP-rP1 (Insulin-like Growth Factor Binding Protein-related Protein 1) is one type of proteins in IGFBPs (insulin-like growth factor binding protein system) which have relatively low binding with IGF (Insulin-like Growth Factor) [4]. Recent studies have shown that IGFBP-rP1 is not only involved in the formation of insulin resistance, but also plays a role in cell growth, aging and tumor formation. It is reported that IGFBP-rP1 can inhibit the growth of tumor cells such as breast cancer, lung cancer, malignant melanoma and colon cancer, and promote the apoptosis of tumor cells [5-8]. The use of IGFBP-rP1 in the transplant model of mice can slow tumor growth and inhibit tumor metastasis [7,9]. IGFBP-rP1 can also alter chemotherapeutic drug sensitivity [6,10]. Furthermore, IGFBP-rP1 exhibited low expression in breast cancer [11], colon cancer [12], prostate cancer [13] and other tumor tissue, indicating that *IGFBP-rP1* might play a role as a tumor suppressor gene. Many studies show that IGFBP-rP1 inhibiting tumor might be related to the regulation of BRAF/MEK/ERK pathway. However, the role of IGFBP-rP1 in EC is still unclear. Previous studies have shown that low levels of IGFBP-rP1 and hyperinsulinemia in serum of patients with EC are risk factors for the formation of EC [14], and an improvement in insulin resistance or metabolic abnormalities can reduce the risk of EC. Therefore, IGFBP-rP1 may play a role in the formation of EC, similar to its role in other tumors, implying a potential therapeutic value for the anti-tumor treatment in EC.

In this study, we up-regulated IGFBP-rP1 at the cellular level first, and then observed its effect on the growth of endometrial carcinoma cells and investigated its regulatory mechanism. Finally, we confirmed the effect of IGFBP-rP1 on the growth regulation of EC on the tissue level.

Methods

Cell lines, transfection, plasmids and reagents

The human endometrial cancer cells HEC-1A were obtained from People's Hospital of Beijing University, China. The cells were cultured in Dulbecco's modified eagle medium (DMEM; Solarbio, China), supplemented with 10% fetal bovine serum and incubated under 5% CO_2 at 37°C.

The plasmids contained the whole IGFBP-rP1 coding region (pEX-2-IGFBP7) and the negative control sequences (pEX-2-Empty) were designed and synthesized by Shanghai Jima Pharmaceutical Technology Company.

Cells were plated in a 6-well plate culture flask with 3×10^5 cells per well. After 20 h, Lipo2000 (Invitrogen, USA) was used as a transfection reagent according to the manufacturer's instructions. The cells transferred with pEX-2-IGFBP7 or pEX-2-Empty were named as the experiment and negative control group, respectively. After 24 h from transfection, the experiment group, negative control group and their parental were collected to undergo cell proliferation, cell cycle and cell apoptosis analysis, respectively. After 36 h or 72 h from transfection, the three groups were collected to perform RNA or protein extraction, respectively.

RNA extraction and real-time PCR

Total RNA was isolated from cells and tissues using the TRIzol reagent (Cwbiotech, China) according to the manufacturer's protocol. cDNA was synthesized by First Strand cDNA Synthesis Kit (Cwbiotech, China) according to the description of instruction. Real-time PCR was performed with SYBR Premix Ex TaqTM (TOYOBO, Japan) as the manufacturer's protocol. The following protocol was used for real-time PCR amplification: preincubated at 95°C for 60 s followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. All the primers used in real-time PCR were described as follows: IGFBP-rP1 (forward primer 5'-CAGGTGTACTTGAGCTGTGAGG-3', reverse primer 5'-CATGTAAGGCATCAACCACTGT-3'); β-actin (forward primer 5'-TGACGTGGACATCCGCAAAG-3', reverse primer: 5'-CAGGAAGGTGGACAGCGAGG -3'). Data were analysed by the 2^{-DDCt} method.

Western blot

This study used antibodies description as follows: IGFBP-rP1 (Abcam), ERK1/2 (Cell Signaling Technology), p-ERK1/2 (Cell Signaling Technology), GAPDH (Good Here,). Western blot was performed as literature described.

Cell proliferation, cell cycle and cell apoptosis assay

For cell proliferation assay, cells were collected and re-suspended with Dulbecco's modified eagle medium, seeded at 8×10^3 cells/well in 96-well plates and cultured in 100 μl Dulbecco's modified eagle medium. The 10 μl cell counting kit-8 (CCK-8) was added to each well after being incubated for the indicated times (12, 24, 48 and 72 h) and then the samples were incubated at 37°C for 2 h and read by 96-well plate reader at 450 nm. All groups were done in triplicate wells.

For cell cycle analysis, cells were collected and fixed in 70% ethanol overnight at 4°C and then treated with the concentration of 10 μg/μl RNAase 2.5 μl for 30 min before with 50 μg/μl propidium iodide and analysed by Fluorescence Activated Cell Sorting (FACS).

Cell apoptosis examination was performed using an annexinV-FITC apoptosis detection kit (Southern Biotech) according to the manufacturer's instruction and then analysed by FACS.

Patients and samples

This study was approved by the Ethics Committee of the first affiliated hospital of Zhengzhou university and followed the Helsinki Declaration. We gathered 45 endometrioid adenocarcinoma, 30 endometrial dysplasia and 30 endometrial simple hyperplasia cases at the first affiliated hospital of Zhengzhou university (Zhengzhou, Henan, China) from October 2013 to May 2014. All cases were selected based on the following criteria: patients had pathologically confirmed diagnosis of EC or not, samples were stored at the pathology department of this hospital; patients were excluded if they underwent neoadjuvant chemotherapy or radiotherapy.

Immunohistochemistry (IHC) and evaluation

The rabbit polyantibody of IGFBP-rP1 was used in IHC to be primary antibody. The IHC was undergoing as the manufacturer's protocol (Cwbiotech, China).

Using the staining results of cytoplasm or cell membrane as the criterion, five high-magnification fields were randomly observed by reference to the semi-quantitative fractionation of positive cells such as Fromowitz, and 100 cells were counted for each field of view.

According to the percentage of positive cells, marking was carried out: <5% for 0 point, 5% to 25% for 1 point, 26% to 50% for 2 points, 51% to 75% for 3 points and >75% for 4 points. According to the positive cell staining intensity, the score was: no colour for 0 point, light yellow for 1 point, brown-yellow for 2 points, brown for 3 points.

According to the total points obtained, the results were categorised as follows: 0-1 point indicates negative (-), 2~3 points indicate weak positive (+), 4~5 points indicate moderate positive (++), 6~7 points indicate strong positive (+++).

DNA methylation assay

According to the manufacturer's protocol, Genomic DNA was isolated from tissues using the Genome DNA extracts kit (Cwbiotech, China) and the DNA methylation was detected by EZ DNA Methylation-gold kit (ZYMO Research, USA). All the primers and annealing temperature of its primer used in PCR amplification were described in Table 1. The PCR products were performed to agarose electrophoresis.

Statistical analyses

Statistical analysis was carried out by means of SPSS (version 17.0; SPSS Inc., Cary, NC, USA). Chi-squared and Student's t-test were used to determine statistical significance at $p<0.05$. The correlation between the DNA methylation and the expression of IGFBP-rP1 in EC was analysed by Spearman coefficients. All data were expressed as and all experiments described above were performed in triplicate.

Results

Efficient up regulation of IGFBP-rP1 in HEC-1A cells

Real-time PCR and Western blot were used to analyse *IGFBP-rP1* gene and protein expression in experiment group, negative control group and their parental, respectively. As shown in Figure 1, the mRNA copy values of IGFBP-rP1 in experiment group, negative control group and their parental were 2.699 ± 0.293, 1.296 ± 0.169 and 1.031 ± 0.301, respectively, which shows that the gene expression of IGFBP-rP1 in experiment group was significantly higher than that in negative control group (P=0.001), whereas no apparent change was observed in negative control group and their parental (P=0.261).

As shown in Figure 2, the relative protein expression levels of IGFBP-rP1 in experiment group, negative control group and their parental were 1.126 ± 0.074, 0.889 ± 0.041 and 0.884 ± 0.042, respectively. They were shown a significantly high lever in experiment group than in negative control group (P<0.01), whereas no apparent change was observed in negative control group and their parental (P>0.05).

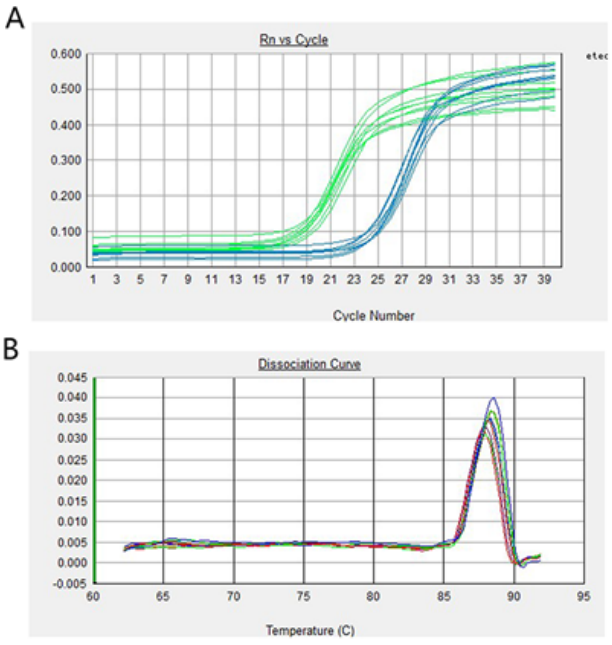

Figure 1. *pEX-2-IGFBP7 containing the whole IGFBP-rP1 coding region increases IGFBP-rP1 mRNA level. (A) Amplification curve revealed the mRNA copy values of IGFBP-rP1 in each group. (B) Melting curve revealed the specificity of the primers.*

IGFBP-rP1 upregulation decreases the cell proliferation, the proportion of cells in the S+G_2/M phase; but increases the cell apoptosis

After 24 h, 48 h and 72 h from transfection, the cellular proliferation inhibition rate in experiment group was 0.373 ± 0.054, 0.399 ± 0.047 and 0.380 ± 0.053, respectively. Correspondingly, the cellular proliferation inhibition rate in negative control group was 0.036 ± 0.006, 0.040 ± 0.005, and 0.0334 ± 0.006, while it was 0.027 ± 0.003, 0.032 ± 0.002 and 0.024 ± 0.002 in parental group. The results showed that the cell proliferation is significantly decreased after IGFBP-rP1 upregulation (P<0.01). However, there was no statistically significant difference between negative control group and parental group (P>0.05).

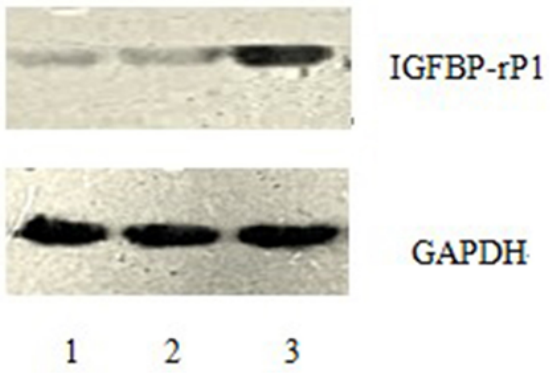

Figure 2. *pEX-2-IGFBP7 containing the whole IGFBP-rP1 coding region increases IGFBP-rP1 protein level. The protein expression levels of IGFBP-rP1 in different groups: line 1 parental; line 2 negative control group; line 3 experiment group.*

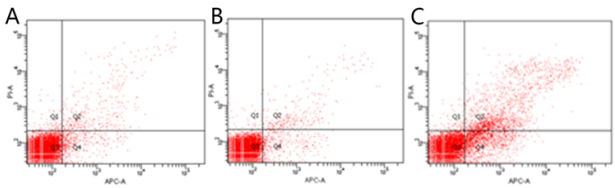

Figure 3. *IGFBP-rP1 upregulation increases the cell apoptosis. The apoptosis in different groups (A) parental group, (B) negative control group, (C) experimental group).*

Twenty-four hours after transfection, the apoptotic rate was 20.667% ± 2.055% in the experimental group, 3.967% ± 0.351% in the negative control group and 4.967% ± 0.252% in the parental group. The experimental group has statistically significant difference in comparison with the negative control group and parental group (P<0.01), but there was no statistically significant difference between the control group and the parental group (P>0.05) (Figure 3). The ratio of S +G_2/M phase cells in the experimental group was 30.980% ± 1.461%, which was significantly lower than that of the negative control group and the parental group (38.797% ± 2.419% and 37.800% ± 0.350%, respectively; P<0.01) There was no statistically significant difference in the cell cycle

between the negative control group and the parental group (P>0.05) (Figure 4).

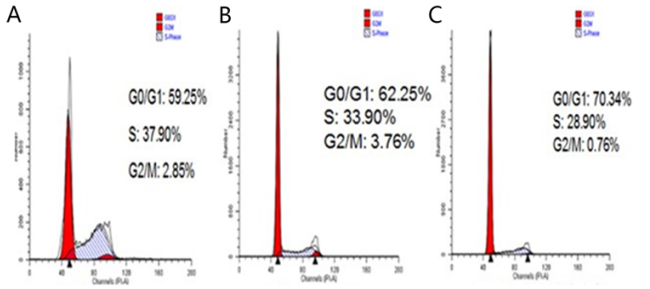

Figure 4. *Effects of IGFBP-rP1 upregulation on the cell cycle of HEC-1A cells by flow cytometry in different groups. (A) Parental group, (B) Negative control group, (C) Experiment group.*

Effects of different concentrations of rhIGFBP-rP1 on proliferation of HEC-1A cells and ERK pathway activation in EC

In the experimental groups, HEC-1A cells were incubated with rhIGFBP-rP1 at concentrations of 2 μg/ml, 4 μg/ml, 8 μg/ml and 16 μg/ml, respectively. After 72 h, compared with the control group, the OD value of each experimental group decreased (the higher the rhIGFBP-rP1 concentration, the more significant the decrease) and cell proliferation was inhibited. Inhibition rate=($OD_{control\ group}$-$OD_{experimental\ group}$)/$OD_{control\ group}$ × 100.

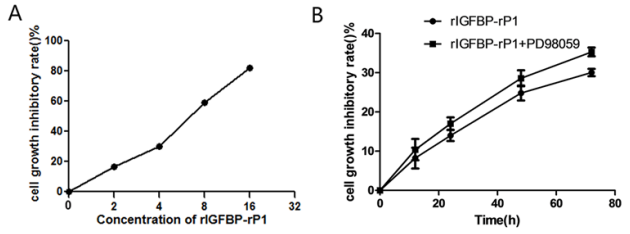

Figure 5. *Effects of different concentrations of rhIGFBP-rP1 or rhIGFBP-rP1 with ERK pathway inhibitor PD98059 on proliferation of HEC-1A cells. (A) Inhibition of proliferation of HEC-1A cells by rhIGFBP-rP1 at different concentrations; (B) 4 μg/ml rhIGFBP-rP1+25 μmol/LPD98059 on HEC-1A cell proliferation inhibition rate.*

As shown in Figure 5A, the inhibitory rates of rhIGFBP-rP1 on HEC-1A were (16.58 ± 1.72%), (30.04 ± 0.94%), (58.74 ± 1.29) and (81.89 ± 0.87%) (P<0.05) for rhIGFBP-rP1 concentration of 5 μg/ml, 4 μg/ml, 8 μg/ml and 16 μg/ ml, respectively. Based on the above data, a statistical analysis using SPSS17.0 obtained IC_{50}=6.39 μg/ml. The inhibitory effect of 4 μg/ml rIGFBP-rP1 on HEC-1A proliferation was more and more obvious with the prolongation of time.

As shown in Figure 5B, the inhibitory rates were (8.25 ± 2.65%), (13.98 ± 1.41%), (24.82 ± 1.90%) and (30.04 ± 0.94%) (P<0.05) for proliferation after 6, 12, 24, 48 and 72 h, respectively. With combination of rhIGFBP-rP1 (4 μg/ml) and ERK pathway inhibitor PD98059 (25 umol/L), the inhibitory rate of cell proliferation increased to (10.04 ± 2.71%), (17.02 ± 1.58%), (28.59 ± 2.04%) and (35.29 ± 1.12%) (P<0.05) for the aforementioned proliferation time.

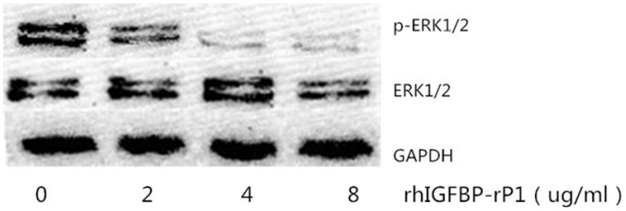

Figure 6. *Expression of total ERK and pERK1/2 protein under different concentrations of rhIGFBP-rP1 by Western blot. The figure shows that the change of total ERK was not evident, while the expression of p-ERK1/2 was inhibited in a dose-dependent manner.*

The change in the expression of total ERK1/2 and p-ERK1/2 protein in the cells was observed by using Western blot after 24 h of treatment with different concentrations of rhIGFBP-rP1 (0 μg/ml, 2 μg/ml, 4 μg/ml, 8 μg/ml). Western blot test showed that the change of total ERK was not evident, while the expression of p-ERK1/2 was inhibited in a dose-dependent manner, as shown in Figure 6.

The expression of IGFBP-rP1 in three endometrial lesions

Tissue immunohistochemistry showed that IGFBP-rP1 was expressed in the cytoplasm and membrane (Figure 7). Overall, a large number of brown stained cells were observed in endometrioid adenocarcinoma tissue, and they were deep stained. There were also many brown stained cells in atypical hyperplasia tissues of endometrium, and the staining was darker. No obvious brown stain was seen in endometrial simple hyperplasia tissue. The positive expression rate of IGFBP-rP1 in endometrial adenocarcinoma (30/45, 66.7%) was significantly higher than that in endometrial dysplasia (5/30, 16.7%) (χ^2=18.080, P=0.00<0.016) and simple endometrial hyperplasia (7/30, 23.3%) (χ^2=13.522, P=0.00<0.016), but there was no statistically significant difference between atypical hyperplasia and simple hyperplasia (χ^2=0.417, P=0.519>0.016).

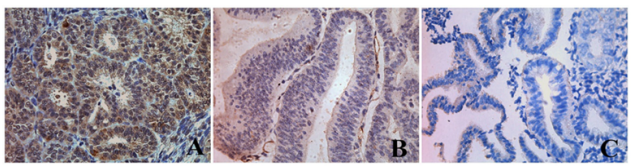

Figure 7. *The expression of IGFBP-rP1 in three endometrial lesions (SP, X400) (A) endometrial adenocarcinoma (B) endometrial dysplasia (C) endometrial simple hyperplasia.*

RT-PCR results showed that the relative expression of IGFBP-rP1 m RNA in endometrioid adenocarcinoma was significantly higher than that in endometrial dysplasia (t=5.17, P<0.01) and simple endometrial hyperplasia (t=6.56, P<0.01), but there was

no significant difference between the first two groups (t=1.33, P>0.05, Table 2).

IGFBP-rP1 gene in the endometrial adenocarcinoma exhibited a methylation status mainly in promoter region, and showed a low methylation status

There were three cases for the methylation status of the *IGFBP-rP1* gene in the tissue: complete methylation, unmethylation and halomethylation (Figure 8), where complete methylation and halomethylation were recorded in methylation.

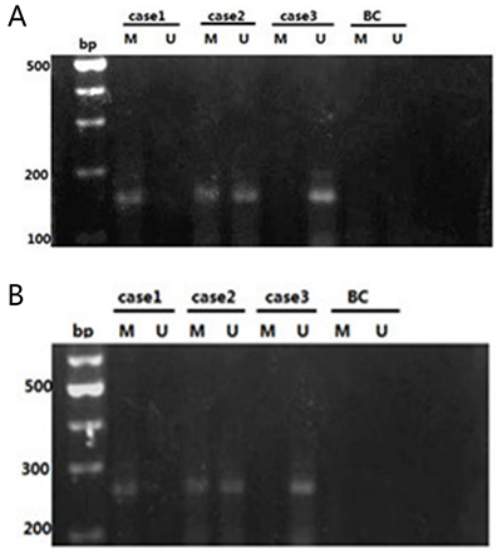

Figure 8. *Methylation status of IGFBP-rP1 gene. (A) Promoter region, (B) First exon region. (M: Methylation; N: Nonmethylation; BC: Blank Control. Case 1: complete methylation status, Case 2: semi methylated state, Case 3: unmethylated state).*

The methylation rate (15/45) of the *IGFBP-rP1* gene in the promoter region of endometrial adenocarcinoma was significantly lower than that in the untypical endometrial dysplasia tissues (20/30) (χ^2=8.036, P=0.005<0.016) and that in the endometrial dysplasia (21/30) (χ^2=9.696, P=0.002<0.016). There was no significant difference in the methylation rate of *IGFBP-rP1* gene in the promoter region between the endometrial hyperplasia tissues and the simple hyperplasia tissues (χ^2=0.077, P=0.781>0.016).

The methylation rate (5/45) of the *IGFBP-rP1* gene in the first exon region of endometrial adenocarcinoma had no significant difference compared with those in the untypical endometrial dysplasia (7/30) and simple hyperplasia (8/30) (χ^2=3.325, P=0.190>0.05).

The methylation rate (15/45) of the *IGFBP-rP1* gene in the promoter region of endometrial adenocarcinoma was higher than that in the first exon region (5/45) (χ^2=6.429, P=0.011<0.05).

IGFBP-rP1 gene was highly expressed in endometrial adenocarcinoma and was negatively correlated with its methylation

In the promoter region, the mRNA expression level of *IGFBP-rP1* gene in methylated endometrial adenocarcinoma was significantly higher than that in non-adimal endometrial adenocarcinoma (t=4.137, P<0.05). The mRNA expression level of endometrial adenocarcinoma in the first exon region was not significantly different from that in the endometrial adenocarcinoma (t=2.123, P>0.05).

In the promoter region, 13 cases showed positive protein expression in 15 cases of endometrial adenocarcinoma with *IGFBP-rP1* gene methylation, which was significantly higher than that of non-methylated endometrial adenocarcinoma. The methylation degree of *IGFBP-rP1* was negatively correlated with protein expression (χ^2=4.050, P<0.05, r=-0.287). In the first exon region, there was no significant difference in the protein expression between the endometrial adenocarcinoma and the non-endometrial adenocarcinoma (t=2.123, P>0.05).

Table 1. *The primers of the IGFBP-rP1 methylation.*

Primer	Sequence	Tm (t/°C)	Product (bp)
Promoter region			
Methylation	F: 5'-AGAAATTAGAGGGTGGAAGAGTCGT-3' R: 5'-CTACTAACGTCGAAAAATAAACGAA-3'	54	173
Nonmethylation	F:5'-AGAAATTAGAGGGTGGAAGAGTTG-3' R: 5'-CTACTAACATCAAAAAATAAACAAA-3'	50	173
First exon region			
Methylation	F: 5'-TTGGGCGAGATTCGCGACGC-3' R: 5'-GACCCTCTAACTAACGACGCG-3'	60	273
Nonmethylation	F: 5'-TTGTTGGGTGAGATTTGTGATGT-3' R: 5'-TCAACCCTCTAACTAACAACACA-3'	54	278

Table 2. *The mRNA levels of IGFBP-rP1 in three endometrial lesions.*

Groups	ΔCT	ΔΔCT	$2^{-\Delta\Delta CT}$
Endometrial simple hyperplasia	6.132 ± 2.390	0.000 ± 2.390	1
Endometrial dysplasia	6.432 ± 1.170	0.300 ± 1.170	0.81
Endometrial adenocarcinoma	2.317 ± 4.372	-3.815 ± 4.372	14.07

Discussion

In recent years, the incidence of endometrial cancer worldwide increased year by year. It is well known that the long-term stimulation of high levels of estrogen without progesterone antagonism is closely related to the occurrence of endometrial cancer, but the mechanism on the pathogenesis of endometrial cancer has not been yet fully understood. Recent studies have found that obesity, hypertension, diabetes, dyslipidemia, and other high risk factors, which collectively constitute metabolic syndrome [15], have become high risk factors for a variety of tumors [16-18]. In particular, the occurrence of endometrial cancer is also closely related to these metabolic disorders [17,18]. The obesity, hypertension and diabetes all have pathophysiological characteristics manifested as insulin resistance and the subsequent hyperinsulinemia [15]. Some recent studies have also found that elevated insulin levels, independent of estradiol, age and hormone replacement treatment, are closely related with endometrial cancer [19].

Insulin-like growth factor system is composed of ligand, cell surface receptor, insulin receptor, IGF binding protein and IGFBP protease composition, and it is the focus in studies of insulin resistance. IGFBP-rP1 is an important member of the IGFBPs family and has been extensively studied because of its low binding to insulin growth factors. Many studies have shown that IGFBP-rP1 plays a role in tumor suppressor genes in various tumors, such as breast cancer [8,20-22], hepatocellular carcinoma [23] and lung cancer [24]; however, it can be expressed as a carcinogenic effect on glioma [25]. Previous investigations showed that IGFBP-rP1 also plays an important role in the neuroendocrine differentiation for certain type of tumor [26]. It has been largely accepted that tumor with neuroendocrine differentiation is one of the most important prognostic factors [27-29]. In the view of the neuroendocrine differentiation, investigations of IGFBP-rP1 in EC have greater significance.

In order to study the role of IGFBP-rP1 in endometrial carcinoma, IGFBP-rP1 cDNA was transfected into endometrial carcinoma cell line HEC-1A with low expression of endogenous IGFBP-rPl. Realtime-PCR and Westem blot confirmed increase in IGFBP-rP1 expression, and then a series of analyses were carried out for the biological function of the IGFBP-rP1 in endometrial cancer cells. The expression of IGFBP-rP1 in endometrial carcinoma cells was significantly increased, the apoptosis rate of endometrial carcinoma cells was significantly decreased, and the apoptosis rate of G0/G1 cells was significantly increased. Such effects are similar to those observed in prostate cancer, breast cancer and lung cancer, etc. [11,13,30-32]. This suggests that IGFBP-rP1 is most likely to play a role in tumor suppression in endometrial cancer cells. In addition, we investigated the mechanism of IGF BP-rP1 suppressing the growth of endometrial carcinoma cells, and found that IGFBP-rP1 reduced the level of phosphorylated ERK1/2 protein by mediating the MEK/ERK signal transduction pathway after insulin receptor, thereby inhibiting endometrial cancer cell proliferation. This function can be enhanced by MEK/ERK signaling pathway inhibitor PD98059. In other words, IGFBP-rP1 can more effectively inhibit endometrial cancer cells HEC-1A with help from the MER/ERK signal pathway inhibitor PD98059.

However, when the inhibitory effect of IGFBP-rP1 was examined using histology, the expression rate of IGFBP-rP1 in endometrioid adenocarcinoma was significantly higher than that in non-typical endometrial dysplasia and simple endometrial dysplasia. These results suggesting that IGFBP-rP1 seems to play a cancer-causing factor on tissue level, which contradicts the results of cell experiments.

The contradictory results of IGFBP-rP1 in the endometrial cancer *in vivo* and *in vitro* suggest that the role of IGFBP-rP1 in endometrial cancer may be pre-transcriptional regulation of genes. Many recent studies have shown that IGFBP-rP1 methylation is correlated with tumor development and progression. *IGFBP-rP1* gene hypermethylation status also appears in a variety of tumors such as laryngeal cancer [33], human melanoma cell line [34], cardia adenocarcinoma [35], gastric cancer cells [36], Barrett esophagus and esophageal squamous cell carcinoma [37], colon cancer [20] and breast cancer [38,39]. These studies have shown that *IGFBP-rP1* hypermethylation state in the promoter region was correlated with its downregulation. In this study, we found that the methylation rate of *IGFBP-rP1* gene in the promoter region of endometrial adenocarcinoma was higher than that in the first exon region, and its abnormal methylation was mainly in the promoter region. Further investigation has shown that the methylation rate of the *IGFBP-rP1* gene in the endometrial adenocarcinoma group was significantly lower in the promoter region than in the non-typical endometrial dysplasia group and the simple endometrial hyperplasia group. The methylation rate of the two latter groups was not statistically significant, and the low methylation level of this gene in the endometrial adenocarcinoma group was negatively correlated with its high expression status. *IGFBP-rP1* gene also showed low methylation levels in colorectal cancer tissues. Lin et al. [20] found that the methylation rate in the first exon region of *IGFBP-rP1* gene was significantly lower in colorectal cancer tissues. There was a negative correlation between the low methylation level and the high expression of the gene in the normal mucosal tissue. The mRNA and protein expressions of *IGFBP-rP1* gene in the endometrial adenocarcinoma and colorectal tissue were significantly higher, suggesting that the expression of this gene regulation occurs at the transcription level or before the molecular events, while DNA abnormal methylation occurs at this level of regulation. This shows the correlation between methylation and expression.

Abnormal methylation of DNA is a common molecular event that occurs in the early stages of human tumors and is a further refinement and development of the Knudson secondary strike mechanism [40]. Once methylation occurs, the transcriptional regulation of the corresponding gene is affected, which inhibits gene expression [41-46]. The present study suggests that the hypermethylation state in the promoter region affects the transcription of the gene, thereby influencing the expression of the gene. This is one of the mechanisms that inhibit the anti-tumor effect of *IGFBP-rP1* gene, which is related to the occurrence and development of tumor. Since DNA methylation is a reversible chemical modification, DNA demethylation may restore the tumor suppressor gene activation and play an anti-tumor effect, which suggests that this principle can be used in clinical anti-tumor treatment, providing new solution to treat cancer.

In summary, this study first found that upregulation of IGFBP-rP1 in endometrial cancer cells can inhibit cell proliferation by inhibiting MEK/ERK pathway. On the other hand, histological studies have shown that overexpression of IGFBP-rP1 was associated with malignancy of endometrial cancer. Further studies have found that the degree of methylation of IGFBP-rP1 in endometrial carcinoma was reduced, and it was negatively correlated with its expression. These results show that the increase in the expression of mRNA and protein due to the reduction in methylation of *IGFBP-rP1* gene promoter may be a predictor of the degree of malignancy of endometrial cancer. The occurrence of cancer is a complex process and thus in the future a further study of the regulation mechanism of IGFBP-rP1 expression may provide new ideas for clinical diagnosis and treatment of endometrial adenocarcinoma.

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Ethic Approval

This study was approved by the Ethics Committee of the first affiliated hospital of Zhengzhou university and followed the Helsinki Declaration.

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*Correspondence to

Ruixia Guo

Department of Obstetrics and Gynecology

The First Affiliated Hospital of Zhengzhou University

PR China