Original Article
FOXM1 as a prognostic biomarker promotes endometrial cancer progression via transactivation of SLC27A2 expression

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Abstract: Endometrial cancer (EC) is one of the most important gynecological cancers, but its pathogenesis is not clearly understood, and it also lacks an effective treatment. The nuclear transcriptional protein forkhead box protein M1 (FOXM1) has crucial functions in the development and progression of cancer and is treated as a prognostic biomarker and therapeutic target in many types of cancers. However, the situation and underlying mechanisms of FOXM1's involvement in EC is largely underestimated. In our present study, we found FOXM1 was overexpressed in EC, including endometrioid (EEC) and serous (SEC). High expression of FOXM1 was meaningfully associated with a poor prognosis of EC patients as well as with EC pathological stages and clinical grades. Knocking down FOXM1 could significantly reduce the proliferation and migration capacity of AN3CA and ISHIKAWA cells. Furthermore, our RNA-seq results indicated that the knockdown of FOXM1 mainly affects downstream metabolic genes in EC cells. Finally, we also discovered one potential functional pathway, FOXM1-SLC27A2, which may contribute to EC progression. Taken together, the high expression of FOXM1 is closely associated with the prognosis, pathological stages, and clinical grades of EC patients. FOXM1 can promote the proliferation and migration of EC cells. Through SLC27A2, FOXM1 may influence the metabolic activity of EC cells, and FOXM1-SLC27A signaling could be treated as a potential cellular target for a therapeutic strategy of EC.

Keywords: Endometrial cancer, FOXM1, prognosis, RNA-SEQ, SLC27A2

Introduction

Endometrial cancer (EC) is one of the three most commonly diagnosed gynecological malignancies, accounting for 4.8% of all cancer and 2.1% of all tumor-associated mortalities among women worldwide [1]. More than 63,000 new patients and over 21,000 deaths were identified for EC in China [2]. Despite the emergence of several new treatments, the prognosis of patients with recurrent or advanced EC remains poor [3, 4]. It is urgent to understand the molecular and genetic events behind EC carcinogenesis. At present, according to the classical Bokhman two element classification, EC is divided into 2 types: type I is the endometrioid (EEC) type, and type II is the non-endometrioid type, in which serous (SEC) is the most common part [5]. Type I EC, EEC is the most common type of EC accounting for about 80% of all new cases and is defined as low grade and stage, noninvasive, well differentiated, estrogen/progesterone receptor positive, and usually with higher survival rate because of the early diagnosis and treatment. Type II EC, including SEC, in general is identified as advanced grade and stage, highly invasive, poor histological differentiation, estrogen/progesterone receptor negative, and with poor prognosis [6, 7]. Although EC frequently arises in the postmenopausal period, 14% of patients are detected in premenopausal women, and 5% of these cases are less than 40 years old or even younger [8]. Women with obesity/overweight are

strongly correlated with EC and account for around 40% of EC incidence in Europe [9]. Indeed, it has been reported that EEC is positively associated with metabolic disorders, such as obesity, diabetes mellitus (types I and II), and dyslipidemia [10]. The metabolic disorders indicate worse control of blood glucose and fatty acids which are all the crucial factors contributing to the development and progression of cancers including EC and other chronic diseases [11]. However, how these metabolic disorders promote the initiation and progression of EC is still unknown.

The forkhead box (FOX) protein family is a large family of transcriptional factors, which plays important functions in many biological processes, such as cell proliferation, cycle regulation, cell differentiation, embryo development, and so on. There are 50 genome members identified in humans. Based on the highly conserved forkhead DNA domain in the structure of the FOX family, the FOX protein can be divided into 19 subclasses which have their own functions [12-17]. By analyzing the GEO database (GSE63678), one finds that there are 21 members of the FOX family which have been found to be expressed in EC cells, including FOXP3, FOXO4, FOXO3, FOXO1, FOXN3, FOXM1, and so on [18]. Forkhead box M1 (FOXM1) has been identified as an anti-cancer target, due to its critical functions on the regulation of mitosis, cell cycle transition and cytokine generation, as well as other leading signal pathways of carcinogenesis [15, 19, 20]. FOXM1 is overexpressed in various cancers, and its high expression is correlated with specific aggressive features, including more cancer cell numbers, large cancer size, and poor differentiation, as well as advanced stages [21, 22]. Overall, FOXM1 eventually influences the prognosis of cancer patients. Importantly, Cui et al. have demonstrated that FOXM1 has critical functions on aerobic glycolysis and tumorigenesis in patients with pancreatic cancer [23]. However, nothing was known about the expression and cellular function of FOXM1 in EC.

To clarify the expression of FOXM1 in EC and the relationship between its expression level and clinical pathological features, its epidemiology, and the prognosis of EC patients, we analyzed EC data from *The Cancer Genome Atlas*

(TCGA) and the *Gene Expression Omnibus* (GEO) and found FOXM1 was highly overexpressed in EC patients. And by utilizing a tissue microarray of our EC patients, IHC indicated that FOXM1 expression was closely related with clinical stages and pathological grades. CCK8 and cell migration assays showed that knockdown of FOXM1 significantly reduced the cell proliferation and migration of EC cells. Combined with siRNA, RNA-seq and other biochemical techniques, we found one novel FOXM1 signal pathway through the downstream protein SLC27A2, which suggested that FOXM1 could be treated as one attractive prognostic biomarker, and its novel signal offered a potential therapeutic strategy for EC.

## Materials and methods

Cell cultures

The human EC cell lines AN3CA, Ishikawa, HEC1A, HECE1B and KLE were all verified and preserved at the Shanghai Cancer Institute. All cell lines were cultured in the DMEM-F12 culture medium (Invitrogen, Carlsbad, CA, USA) according to ATCC protocols and supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen) and 1% antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin, Invitrogen) in a humidified incubator at 37°C with 5% CO_2.

Clinical samples and database

In all, 137 clinical samples and their corresponding followed-up information, including 120 EC samples and 17 non-cancerous endometrial samples, were collected by the Department of Gynecology and Obstetrics of Shanghai Jiao Tong University Affiliated Sixth People's Hospital, of Fengxian Central Hospital, Shanghai and The First People's Hospital of Huai'an City, Jiangsu province, as well as Changning Central Hospital, Shanghai between April 2006 and March 2013. All the samples underwent tissue resection and the patients signed informed consents. All procedures were performed in accordance with the human investigation ethical review committees of the above hospitals.

To investigate how FOXM1 expression changes in EC and non-EC patients, we downloaded and analyzed the EC corpus in *The Cancer Genome Atlas* (TCGA, https://cancergenome.nih.gov/)
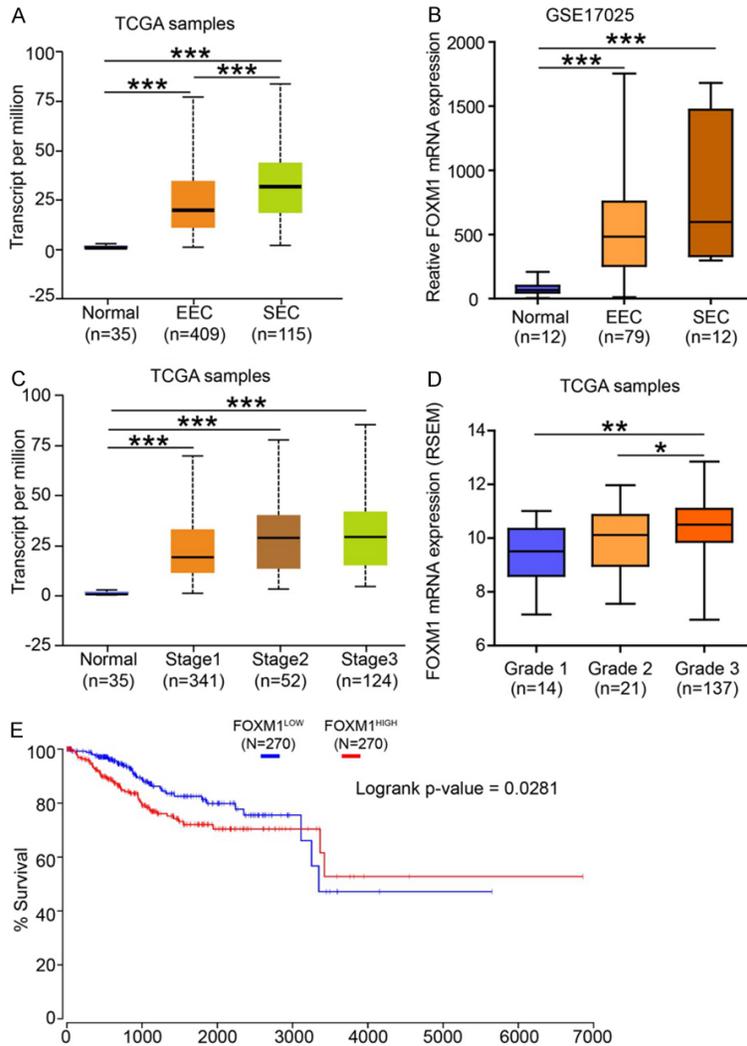
Figure 1. Analysis of FOXM1 expression in EC tissues using TCGA and GEO databases. A. Analysis of FOXM1 mRNA expression in different types of EC from the TCGA database. B. FOXM1 expression in EC microarray from GEO database GSE17025. C, D. Analysis of FOXM1 mRNA expression in different pathological stages and clinical grades of EC compared to normal endometrium tissue from the TCGA database. E. A Kaplan-Meier analysis of overall survival according to FOXM1 expression in 540 EC patients. The *P* value was calculated by a log-rank test. *P<0.05, **P<0.01, ***P<0.001.

UCAAGAUUAUUAUT-3'; siNA1-R 5'-UAAUAAUCUUGAUCCCA-GCTT-3'; siRNA2-F, 5'-GGCU-GCACUAUCAACAAUATT-3'; siR-NA2-R, 5'-UAUUGUUGAUAGU-GCAGCCTT-3'; Negative control siRNA-F, 5'-UUCUCCGAA-CGUGUCACGUTT-3'; Negative control siRNA-R, 5'-ACGUGAC-ACGUUCGGAGAATT-3'.
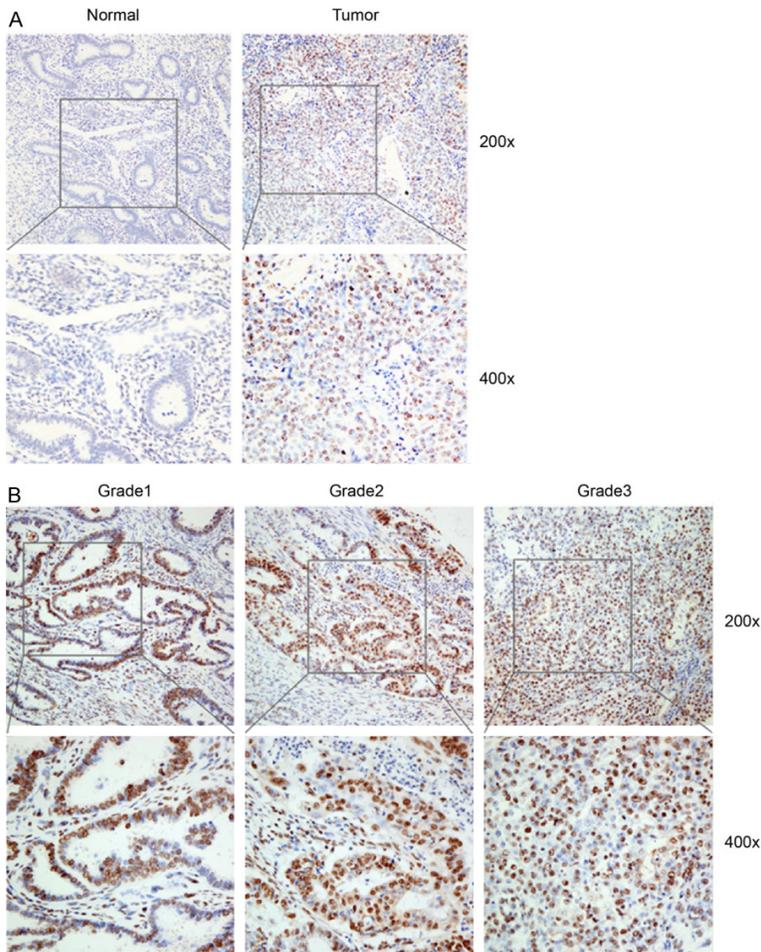
Immunohistochemistry (IHC)

All tissue samples were fixed in phosphate-buffered neutral formalin and routinely embedded in paraffin, and then cut into 4-μm-thick sections. The sections were first deparaffinized with xylene and rehydrated and followed by antigenic retrieval. The slides were incubated with 3% H_2O_2 in PBS for 30 min and blocked by 10% BSA (Sangon, Shanghai, China) for 1 hour, and further detected by the anti-FOXM1 (1: 1000, ab207298, Abcam) primary antibody overnight at 4°C in a moist chamber. After incubation with the secondary antibody, HRP-conjugated goat anti-rabbit IgG (1: 2000, CST) labeled with HRP (rabbit) for 1 hour at room temperature, the slides were treated with diaminobenzidine and counterstained with hematoxylin. The FOXM1 expression was identified as positive or negative expression by two pathologists independently.

and in GSE17025 from *The Gene Expression Omnibus* (GEO, http://www.ncbi.nlm.nih.gov/geo/).

Cell transfection and RNA interference

Different siRNA oligos were ordered from Gene Pharma (Shanghai, China). The cells were transfected with siRNA oligos using Lipofectamine RNAi MAX (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The sequences of FOXM siRNA are, siRNA1-F, 5'-GCUGGGGA-

Quantitative real-time PCR (qPCR)

Total RNA from the EC cell lines was extracted using Trizol (Takara) according to the manufacturer's instructions. cDNA was reversed transcribed using a PrimeScript RT-PCR Kit (Takara). Real-time PCR analyses were performed with SYBR Premix Ex Taq (Takara Bio, Inc.) on a 7300 Real-time PCR system (Applied Biosystems) at the recommended thermal cycling settings: one initial cycle at 95°C for 30 s followed by 40 cycles of 5 sec at 95°C and 31 s at

Figure 2. Immunohistochemical expression of FOXM1 in EC tissues. A. Representative IHC images of FOXM1 expression in EC samples and normal endometrium samples. B. Representative IHC images of FOXM1 expression in different grades of EC samples.

60°C. The mRNA level of FOXM1 was normalized to the GAPDH mRNA level. Primer sequences used in our study were as follows: FOXM1-F, 5'-ATACGTGGATTGAGGACCACT-3'; FOXM1-R, 5'-TCCAATGTCAAGTAGCGGTTG-3'; GAPDH-F, 5'-GCACCGTCAAGGCTGAGAAC-3'; GAPDH-R, 5'-ATGGTGGTGAAGACGCCAGT-3'. The relative expression was calculated using the 2-ΔCT method.

Western blot

The proteins were extracted using a total protein extraction buffer (New Cell & Molecular, China) supplemented with a 1X protease and phosphatase inhibitor cocktail (New Cell & Molecular, China). The proteins were separated by SDS/PAGE under reducing conditions and transferred to the NC (or PV-DF) membrane, followed by blocking with 1% BSA. The primary and secondary antibodies used were as follows: anti-FOXM1 (1:1000, ab207298), anti-SLC27A2 (1:1000, ab22-8784), anti-ALDH2 (1:500, ab-108306), anti-β actin (1:5000, CWBIO, China); HRP-conjugated goat anti-rabbit IgG (1:20-00, CST), HRP-conjugated goat anti-mouse IgG (1:5000, CST). The bound antibodies were visualized using the Lumi Best ECL reagent solution kit (Share-Bio, China).

CCK8 assay

AN3CA and Ishikawa cells with/without FOXM1 knockdown were seeded into a 96-well plate at 4,000 cells/well with a 100 µl complete medium and cultured at 37°C. A total of 10 µl Cell Counting Kit-8 (CCK-8) (WST-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) solution in 90 µl medium was added to each well after 0, 1, 2, 3, and 4 days, respectively. After 1 h of incubation, WST-8 was metabolized to produce a colorimetric dye that was detected at OD_{450} nm by using a PowerWave XS microplate reader (BioTek Instruments, Inc.). The experiment was performed in triplicate and repeated twice.

Cell migration assay

The migrative potential of the cells was measured using 8 µm pore size cell culture inserts within a 24-well plate (Corning, USA), according to the manufacturer's instructions. 2×10^4 AN3CA and Ishikawa cells with/without FOXM1 knock down in 200 µl medium were seeded into the upper chambers of the transwell (Millipore, USA). The cells that had migrated were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The number of cells on the lower surface was counted under a light micro-

Table 1. Comparison of FOXM1 expression between EC and normal endometrium tissues

	FOXM1		Total	X^2	P-value
	Negative	Positive			
EC	62	58	120	10.94	<0.001***
Normal	16	1	17		

***P<0.001.

Table 2. The relationship between FOXM1 expression and EC grades

EC grades	FOXM1		Total	X^2	P-value
	Negative	Positive			
G1-2	54	33	87	13.71	<0.001***
G3	8	25	33		

***P<0.001.

Table 3. The relationship between FOXM1 expression and different pathological stages of EC

EC stages	FOXM1		Total	X^2	P-value
	Negative	Positive			
I-II	56	44	100	4.51	<0.05*
III	6	14	20		

*P<0.05.

scope in six random fields. Each experiment was performed in triplicate and repeated twice.

RNA-Sequencing and gene expression analysis

To identify the genes regulated by FOXM1, RNA-seq was performed for three repeats of knocked down FOXM1 and the negative control in AN3CA and Ishikawa, respectively. Total RNAs of cell samples were isolated using the TRIzol reagent for RNA sequencing following the manufacturer's instructions. The construction of the libraries and their qualities were checked using an Agilent 2100 Bioanalyzer (Agilent). The library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). The clustering of the index-coded samples was performed on a cBot Cluster Generation according to the manufacturer's instructions (TruSeq PE Cluster Kit v3-cBot-HS, Illumia). After cluster generation, the library preparations were sequenced on an Illumina Hiseq 2500 and 150 bp paired-end reads were generated. HTSeq v0.6.0 was used to count the read numbers mapped to each gene. Each

gene was calculated as FPKM based on the length of the gene and the read count mapped to each gene.

After receiving all the FPKM values of the genes, differentially expressed genes from the comparisons between FOXM1 siRNAs and the negative controls were generated using a 2-fold cut-off with Benjamin-Hochberg adjusted *P* values less than 0.05 by unpaired T-test. Furthermore, FOXM1-dependent genes were defined by identifying the cross-set of these two cell lines.

Statistical analysis

All statistical evaluations and graphical representations were performed with Graph Pad Prism 7 (San Diego, CA) software. Data were presented as mean ± SD. Student's t-test and chi-square test were used for comparisons between groups. P<0.05 was considered statistically significant.

Results

FOXM1 expression was significantly increased in EC and positively correlated with EC clinical grades and pathological stages

To illustrate the expression pattern of FOXM1, we first investigated FOXM1 expression in EC by TCGA analysis. The data showed that FOXM1 mRNA levels in both EEC and SEC were significantly up-regulated compared to the normal endometrium tissue and the FOXM1 mRNA levels in SEC were much higher than that in EEC (**Figure 1A**). Similar results were obtained from the GEO dataset (GSE17025) (**Figure 1B**). Furthermore, the mRNA levels of FOXM1 were positively correlated with EC clinical stages and pathological grades (**Figure 1C**, **1D**). Additionally, the Kaplan Meier analysis based on the TCGA cohort indicated that the patients with high expression levels of FOXM1 exhibited a significantly worse overall survival ratio than th-
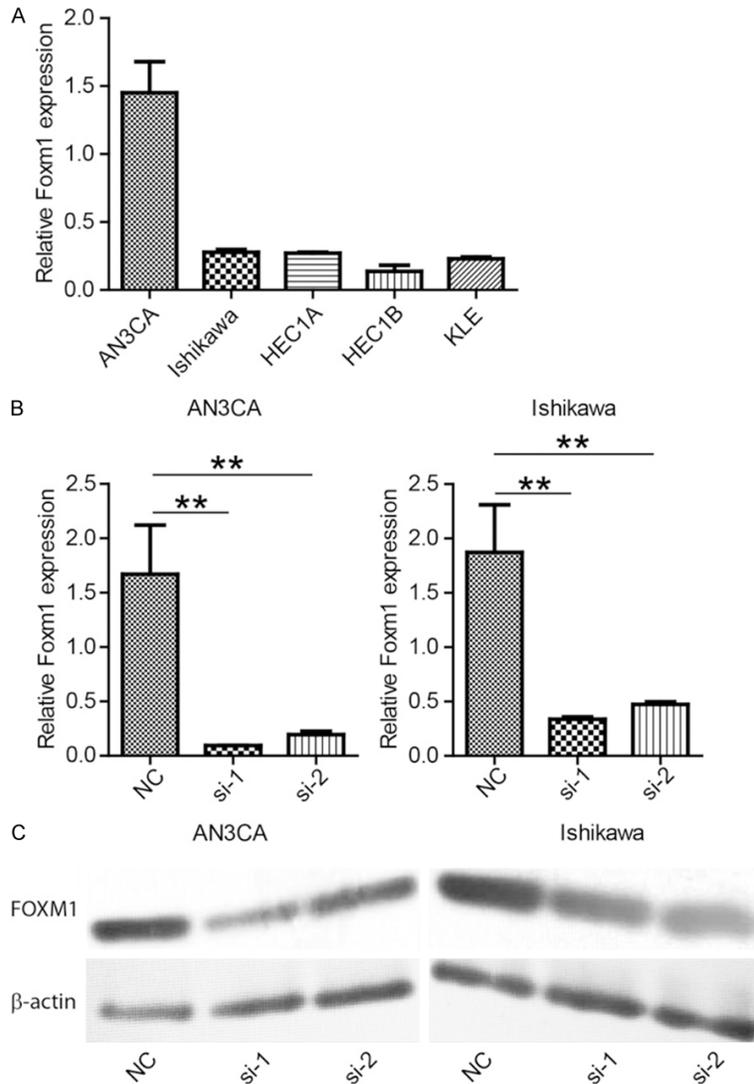
Figure 3. FOXM1 expression in EC cell lines. (A) The FOXM1 mRNA expression in 5 different EC cell lines measured by Real-Time PCR. (B, C) The FOXM1 knockdown was confirmed by Real-time PCR (B) and WB (C) in AN3CA and Ishikawa EC cell lines.

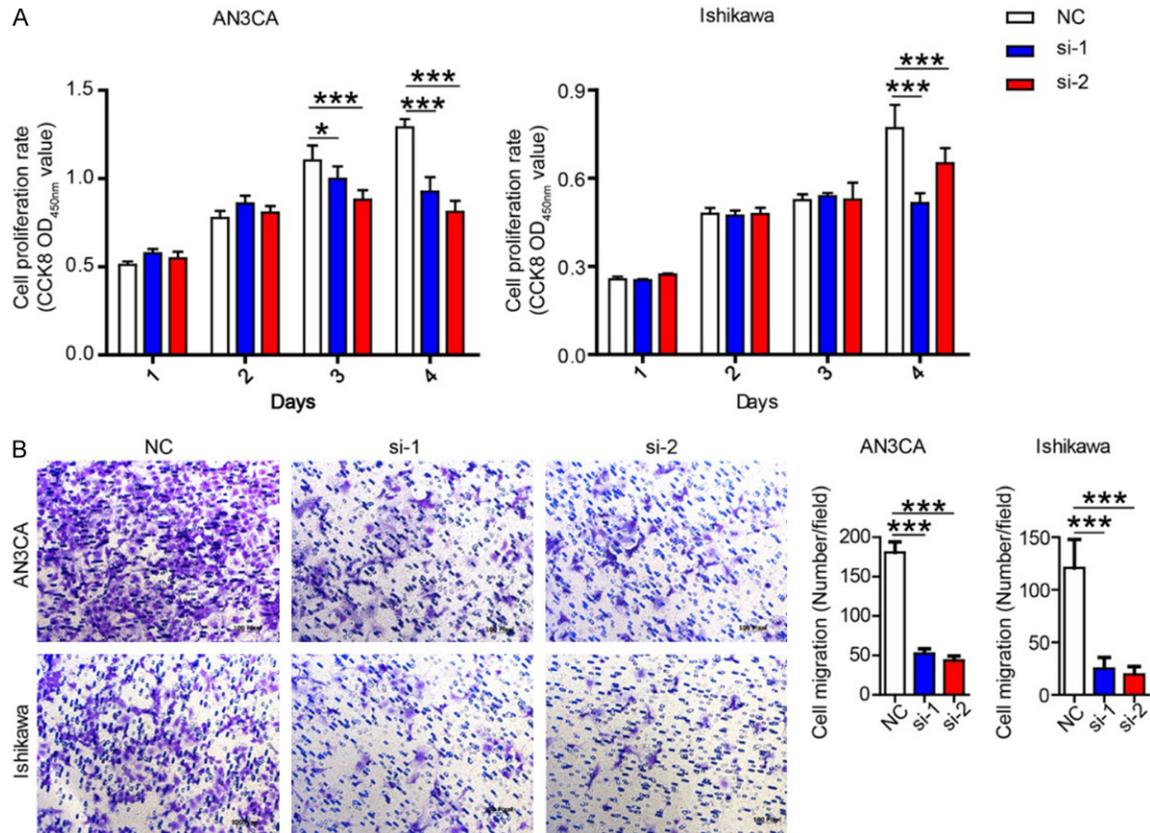showing a significant difference between the EC and normal endometrium groups (P<0.001) (**Table 1**).

Next, we analyzed the relationship between FOXM1 expression and EC grades or different EC pathological stages. **Figure 2B** showed a positive correlation between protein levels of FOXM1 and different EC grades (**Figure 2B**). Statistically, 38% (33/87) of the Grade 1-2 EC samples displayed positive FOXM1 expression, and this number was increased to 76% (25/33) in Grade 3 EC, which showed a significant difference (P<0.001) (**Table 2**). For different pathological stages of EC, 44% (44/100) of stage I-II patients exhibited FOXM1 positive staining, while FOXM1 positive staining was seen in 70% (14/20) in stage III of EC, which pointed out that the FOXM1 expression level was also significantly different between EC stages I-II and stage III (P<0.05) (**Table 3**).

FOXM1 promoted EC cell proliferation and migration in vitro

Real-Time PCR analysis showed that various mRNA levels of FOXM1 in 5 cell lines that were all derived from advanced EC (AN3CA, HEC1A, HECE1B, Ishikawa, KLE, **Figure 3A**). Among them, AN3CA and Ishikawa with a relatively high level of FOXM1 mRNA were selected for the construction of FOXM1-silenced cell lines, to identify the biological functions of FOXM1 in EC. Knockdown was confirmed in both the mRNA and protein levels (**Figure 3B**, **3C**).

We first examined the effect of FOXM1 knockdown on EC cell growth. A CCK8 assay showed that the knockdown of FOXM1 significantly suppressed the proliferation of AN3CA cells after 3 days and Ishikawa cells after 4 days (**Figure**

ose with lower expression levels of FOXM1 (P<0.05) (**Figure 1E**).

To further verify the FOXM1 protein changes in the EC clinical samples, an immunohistochemical analysis was performed on the 17 normal endometrium tissues and the 120 EC tissues. The results revealed that FOXM1 staining was stronger in the EC tissues than in the normal endometrium tissues (**Figure 2A**). Furthermore, FOXM1 positive expression was detected in 48% (58/120) of the EC clinical samples, but in only 1 out of 17 (0.06%) of the normal endometrium tissues showed positive FOXM1 staining,

Figure 4. Knockdown of FOXM1 promoted EC cell proliferation and migration in vitro. A. Cell proliferation analysis in AN3CA (left) and Ishikawa (right) cells with or without knockdown of FOXM1, as measured by a CCK-8 assay. n = 6. Values are means ± SEM. B. Left: Representative images of AN3CA and Ishikawa cell migration with or without FOXM1 knock down. Right: The quantification of migration was performed from 6 randomly selected fields.

4A). Next, we investigated the effects of FOXM1 on EC cell migration in vitro. The results showed that the knockdown of FOXM1 significantly inhibited AN3CA and Ishikawa cell migration in vitro (**Figure 4B**).

A majority of FOXM1 regulated genes involved in metabolism

To discover the gene profiles which were regulated by FOXM1, RNA-seqs were performed. In total, 14 genes were detected after the specific knockdown of FOXM1 with individual siRNA in 3 biological repeats in AN3CA and Ishikawa, respectively (**Figure 5A**, **5B**). The unsupervised cluster results presented in **Figure 5A** were secondary proof of our knockdown experiments. Intriguingly, the majority of the down-regulated genes were involved in metabolism, which indicated that FOXM1 may affect the metabolic activity of EC cells, not the cell cycle checkpoint.

FOXM1 has a potential effect on EC cell metabolic activity by interacting with SLC27A2

On the mRNA level, we identified 6 novel down-regulated candidates regulated by FOXM1 by an RNA-seq assay (**Figures 5B**, **6A**). To further confirm these interactions on the protein level, we chose SLC27A2 and ALDH2 for a Western blot analysis after FOXM1 knowndown. It has been reported that SLC27A2 has an important role in the chemotherapeutic drug resistance of ovarian cancer cells [24] and lung cancer cells [25]. Previous studies have pointed out that ALDH2 was involved in a wide variety of digestive [26] and ovarian cancers [27]. The results showed that only the protein level of SLC27A2 existed in the corresponding down-regulated AN3CA and Ishikawa cells, not the protein level of ALDH2 (**Figure 5B**). Taken together, our study demonstrated that FOXM1, by modulating both the gene and protein levels of SLC27A2, may affect EC cells metabolic or chemo-resistance
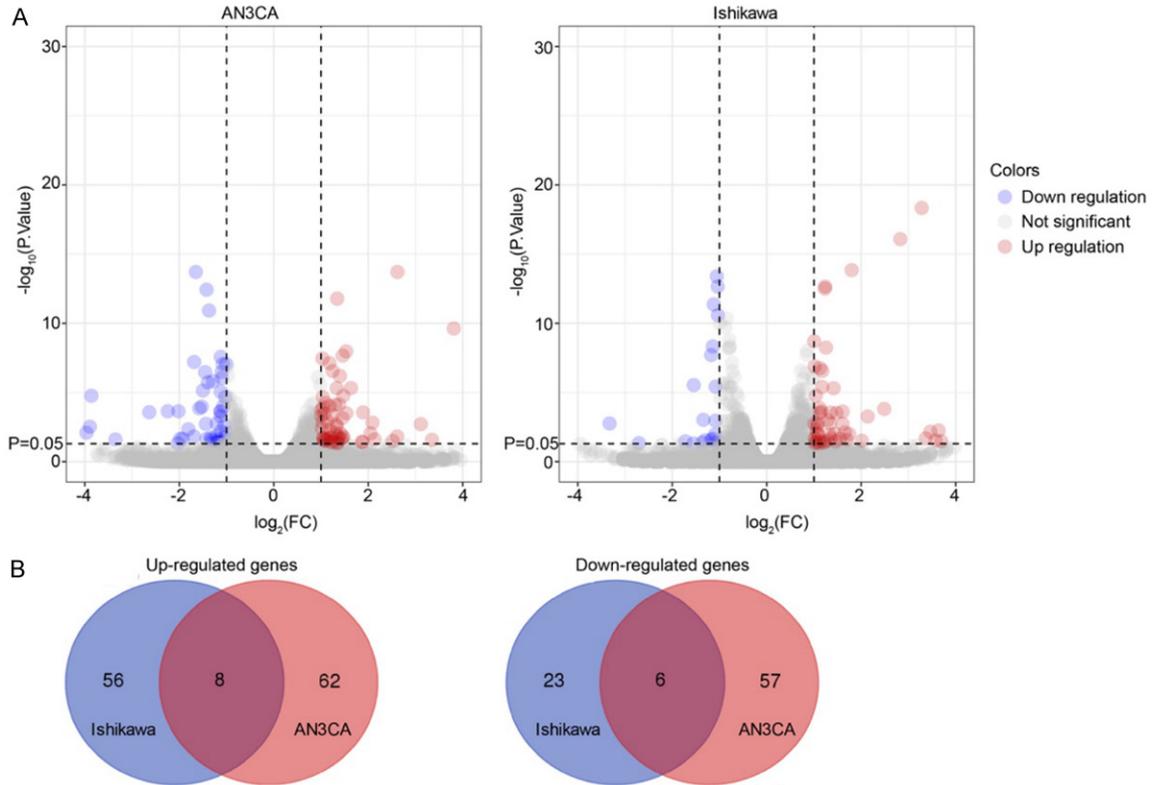
Figure 5. The differential expression genes were detected between normal and FOXM1 knocked down EC cell lines using RNA-seq analysis. A. Volcano plot profiles showing the differential expression genes in AN3CA (left) and Ishikawa (right) EC cells lines. The blue plots show down-regulated genes, the gray ones show non-significantly expressed genes, and the red ones show up-regulated genes. $P<0.05$, $|\log_2(FC)|>1$. B. Venn plots showing the up-regulated genes (left) and the down-regulated genes in both the Ishikawa and AN3CA EC cell lines after FOXM1 knock down.

activities, which needs further cellular function investigation.

Discussion

Global cancer statistics have shown that the number of new cases and deaths from EC are increasing every year worldwide. In the United States, the estimated numbers of new cases and deaths in 2017 were 61,380 and 10,920, respectively [28]. Although the early stages of EC can be easily detected, the prognosis of patients with recurrent or advanced endometrial cancer remains poor. It is already well known that histological factors could affect the prognosis of EC, including clinical stage, histological grade, depth of invasion, lymph vascular space invasion (LVSI), and histological subtype [3, 4]. This is consistent with the gynecological cancer report by FIGO in 2015. However, EC patients with similar clinical and pathological features have different outcomes. In addition,

the physical examination of relapsed patients has become difficult due to local anatomic changes which are induced by initial treatment, so the physical examination can only rely on CT/MRI. However, frequent CT or MRI examinations are costly, so specific biomarkers closely associated with clinical features and prognosis of EC are urgently needed. Several bio-factors have already been used for the prognosis of EC, such as PR, ER, CA125, P53, Ki-67, C-erbB-2, and HE4, but none of them is specific or has any constructive value for the prediction and screening of EC.

Among the histological factors of EC, the clinical stage is the most important prognostic factor, and staging has a very high value for the comprehensive estimation of EC patients' outcomes. The second most important factor is pathological grade, which represents the relationship between the degree of tissue differentiation and prognosis. Narayan et al. [29]
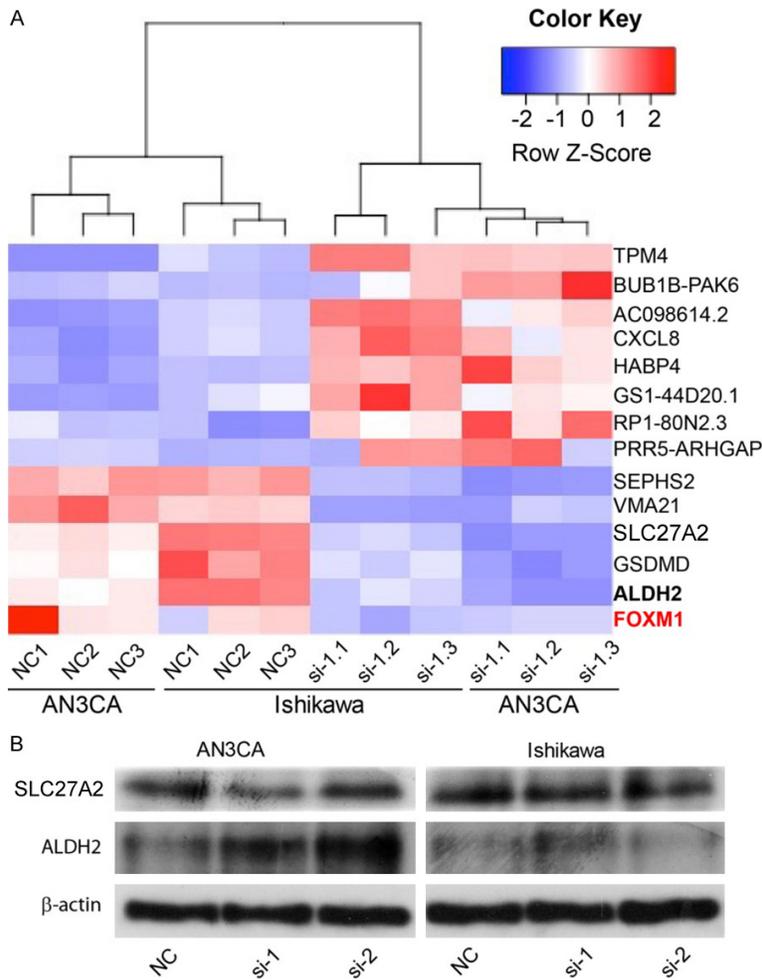
Figure 6. The expression of SLC27A2 was correlated with FOXM1 expression in 2 EC cell lines. A. The heat map analysis of RNA-seq results showing the 8 up-regulated genes and 6 down-regulated genes in the FOXM1 knockdown AN3CA and the Ishikawa cells compared to the control cells. B. The expression of SLC27A2 and ALDH2 were detected in FOXM1 knockdown cells compared to the control cells. β-actin was used as an internal control.

functional regions: one is the N-terminal autonomous inhibitory domain (NRD), and the second is the DNA binding region, which is called "Forkhead" or Winged-helix domain (FKH); the third one is the c-terminal transcriptional activation domain (TAD). FOXM1 is commonly expressed during the development of embryos and significantly downregulated at the end of differentiation. In adults, FOXM1 is usually detected in organs containing more proliferating cells, such as the thymus, testis, small intestine, and colon. It is also additionally expressed in circulating cells and plays important roles in cell proliferation and DNA breakage repair.

Recently, it has been reported that FOXM1, as one oncogenic transcription factor, is overexpressed in various of cancers, such as lung cancer [30], breast cancer [31], liver cancer [32], pancreatic [33] and gastric cancer [34], and its expression is closely correlated with the progression and prognosis of these diseases. Furthermore, it is also involved in carcinogenesis, epithelial-mesenchymal transition, invasion and metastasis [19]. Wen et al. [35] reported that FOXM1 is overexpressed in ovarian cancer and promotes proliferation, invasion and distant metastasis of ovarian cancer cells, resulting in a poor prognosis. In esophageal cancer [36], FOXM1 can upregulate the expression of MMPs, especially MMP-2 and MMP-9, and then promote tumor invasion and metastasis. FOXM1 can also promote tumor angiogenesis by enhancing the expression of VEGF, thereby promoting tumor progression [34, 37]. We found the proliferation and invasion capacity of EC cells were significantly inhibited after knocking down FOXM1, which implies that FOXM1 may promote the progression of EC and the potentiality of FOXM1 as a biomarker for the prognosis of EC.

reported that the pathological subtype and histological grade both were prognostic factors in patients with high-risk EC. Histological grade was also closely related to lymph node metastasis. In this study, both the TCGA and GEO databases showed that FOXM1 is highly expressed on EC and its high expression level is associated with poor prognosis of EC. Our histopathologic findings are consistent with the fact that the positive expression of FOXM1 has a significant relationship with the clinical stage and pathological grade of EC.

FOXM1 is a member of the transcription factor forkhead box superfamily. It consists of three

Recent studies have shown that tumor metabolism plays an important role in the development of EC [38, 39]. In our study, after knocking down FOXM1 in two EC cell lines, the majority of the down-regulated genes (shown in **Figure 5A**) were found to be involved in the metabolism pathway, which was in accordance with metabolic disorders, such as glycolysis and lipogenesis, and which could promote the growth of EC [39]. Among the 6 down-regulated genes, SLC27A2 is a transmembrane transporter coenzyme that participates in the long-chain fatty acid beta-oxidation and peroxidase lipid metabolism. ALDH2 has oxidoreductase activity and aldehyde dehydrogenase (NAD) activity. SLC27A2 and ALDH2 not only belong into insulin resistance/fatty acid synthase pathway and glycolysis/gluconeogenesis pathways respectively, but also play critical roles in different types of cancer [24-27]. In addition, Frances et al. have pointed out that glucose metabolism was a key factor for the growth of endometrial carcinoma [39]. Although SEPHS2 and VMA21 are involved in the metabolism pathway, neither of them has been reported in carcinogenesis. GSDMD is mainly found in the inflammation and cell cycle pathways, but, it has been supposedly acting as a cancer suppressor [39]. Due to the above reasons, we only concentrated on the role of FOXM1 in the metabolic activity of EC by regulating SLC27A2 or ALDH2 in our study. WB results showed that only the expression of SLC27A2 was associated with that of FOXM1 in both AN3CA and Ishikawa cells. All these data demonstrated FOXM1 may influence the cellular activity of EC cells by modulating the expression of SLC27A2. In addition, a causal relationship between FOXM1 and chemotherapy resistance has been identified by increasing evidence [39], which may also connect to the drug resistance features of SLC27A2. Although our study indicated the interaction partners and potential functions of FOXM1 in EC, further studies should be carried out to verify the exact cellular effects of FOXM1 in EC cells.

In conclusion, our study not only identified FOXM1 overexpression in EC compared to normal tissues and the positive association between its high expression and clinical pathological features as well as its prognosis, but our study also provided crucial insight into the potential function of FOXM1 in the progression and metabolism of EC, and we discovered one novel FOXM1-SLC27A2 signaling pathway in EC. Together, all of our data suggest that FOXM1 may be an attractive therapeutic target and outstanding prognostic biomarker for EC.

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Disclosure of conflict of interest

None.

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