Estrogen receptor β regulates endometriotic cell survival through serum and glucocorticoid–regulated kinase activation

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Objective: To determine the expression and biological roles of serum and glucocorticoid–regulated kinase (SGK1) in tissues and cells from patients with endometriosis and from healthy control subjects.
Design: Case-control.
Setting: University research setting.
Patient(s): Premenopausal women.
Intervention(s): Endometriotic tissues were obtained from women with ovarian endometriosis, and normal endometrial tissues were obtained from women undergoing hysterectomy for benign conditions.
Main Outcome Measure(s): Expression levels of SGK1, the role of SGK1 in endometriosis pathology, and regulation of SGK1 by estrogen receptor (ER) β.
Result(s): Transcript and protein levels of SGK1 were significantly higher in endometriotic tissues and cells compared with normal endometrium. SGK1 mRNA and protein levels were stimulated by E2, by the ERβ-selective agonist diarylpropionitrile, and by prostaglandin E2. SGK1 was transcriptionally regulated by ERβ based on small interfering RNA knockdown and chromatin immunoprecipitation of ERβ followed by quantitative polymerase chain reaction. SGK1 knockdown led to increased cleavage of poly(ADP-ribose) polymerase, and SGK1 activation was correlated with the phosphorylation of FOXO3a, a proapoptotic factor.
Conclusion(s): ERβ leads to SGK1 overexpression in endometriosis, which contributes to the survival of endometriotic lesions through inhibition of apoptosis. (Fertil Steril® 2016;105: 1266–73. ©2016 by American Society for Reproductive Medicine.)
Key Words: ERβ, SGK1, endometriosis, PGE2, inflammation

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Endometriosis is a debilitating disease that affects 5%–10% of women of reproductive age (1, 2). Clinical manifestations of endometriosis include chronic pelvic pain and infertility; a conclusive diagnosis requires laparoscopy and pathologic examination of the lesions (3, 4). Although the etiology of the disease is unknown, several theories have been proposed to explain the development of endometriosis. These theories include retrograde menstruation, coelomic metaplasia, and vascular/lymphatic dissemination (5–8). Although the theory of retrograde menstruation is the most accepted, the lack of one defined theory highlights the complex nature of the disease.

A genetic basis for endometriosis has not been clearly established; however, among sisters, there is a five-fold increased probability of developing endometriosis (9, 10). Genome-wide association studies have identified chromosomal regions near the coding regions of *HOXA10*, *HOXA11*, *WNT4*, *IL33*, and *IL1A* that are associated with the disease (11–13). Epigenetic defects are also present in endometriosis (14, 15), with hypomethylation present

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in the promoters of several key genes, including *ESR2* (16), *NR5A1* (17), *GATA6* (15), *HOXA10* (18), and *CYP19A1* (19). Hypomethylation of the *ESR2* promoter is associated with increased gene and protein levels in diseased tissues (16). The inflammatory state of endometriosis is also correlated with the levels of estrogen receptor (ER) β in diseased tissues (20). Because of the strong estrogen-mediated effects on the disease and the high expression levels of ERβ in affected tissues, studies are needed to determine ERβ transcriptional targets in endometriosis.

We recently found that ERβ drives the transcription of several genes with altered expression in endometriotic stromal cells (21). One of the genes identified in that screen was the serum and glucocorticoid–regulated kinase (*SGK1*), which is overexpressed in endometriosis and has an ERβ-binding site in its promoter region. The antiapoptotic roles of SGK1 have been described in several studies, which indicate that SGK1 mediates the cell's response to environmental stress (22, 23). SGK1 has also been shown to regulate the proapoptotic factor FOXO3a (24).

In the present study, we tested the hypothesis that SGK1 is a transcriptional target of ERβ and is up-regulated in endometrial tissues to promote endometriotic stromal cell survival. We examined SGK1 expression in tissue retrieved from women with endometriosis and from healthy women undergoing hysterectomy for benign conditions. We also used small interfering (siRNA) knockdown of ERβ, as well as E2 and prostaglandin E2 (PGE2) treatments to examine the regulation of SGK1 and its downstream targets in endometriosis.

MATERIALS AND METHODS

The study was approved by the Northwestern University Institutional Review Board, and informed consents were obtained from all participants (Reproductive Tissue Registry STU00018080).

Isolation and Culture of Primary Human Normal Endometrial and Endometriotic Stromal Cells

Normal endometrium (NoEM) and ovarian endometrioma cyst walls (E-Osis) were used for all experiments. We used freshly isolated tissues or stromal cells within three passages after initial culture. Tissues were obtained during the follicular phase of the menstrual cycle, and women on oral contraceptives or other hormonal therapies were excluded from the study. NoEM was obtained from women undergoing hysterectomies for benign conditions other than endometriosis, and E-Osis was obtained from women undergoing surgical removal of endometriosis. For E-Osis samples, confirmation of endometriosis was obtained from clinical diagnosis and by pathology analysis. For the studies performed in stromal cells, we established primary human cell cultures as previously described (25, 26). Briefly, tissues were digested with the use of a 0.2 mg/mL DNase and 5 mg/mL collagenase solution followed by a second digestion in a 0.2 mg/mL DNase, 5 mg/mL collagenase, 1 mg/mL pronase, and 2 mg/mL hyaluronidase solution prepared in Hanks buffered saline solution. The primary cells were cultured in DMEM/F-12 (Life Technologies) supplemented with 10% fetal bovine serum (FBS) and antibiotic/antimycotic solution (VWR Scientific).

Compounds and Reagents

Estradiol(E2), diarylpropionitrile (DPN), and propyl pyrazole triol (PPT) were purchased from Tocris, resuspended in ethanol, and used at a final concentration of 100 nmol/L. PGE2 was purchased from Cayman, resuspended in ethanol, and used at a final concentration of 50–100 nmol/L. Phosphatase Inhibitor Cocktail (cat. no. P5726) and Protease Inhibitor Cocktails (cat. no. P8340) were purchased from Sigma and diluted 1:100 in the appropriate lysis buffer. The following antibodies were used for immunoblotting: SGK1 (cat. no. 3272; Cell Signaling), cleaved poly(ADP-ribose) polymerase (PARP; cat. no. 9542; Cell Signaling), pFOXO3a (cat. no 9464; Cell Signaling), FOXO3a (cat. no 2497; Cell Signaling), and β-actin (cat. no A1978; Sigma). Anti-SGK1 (cat. no ADI-KAP-PK015-D; Enzo) antibody was used for immunohistochemistry.

Real-time Quantitative Polymerase Chain Reaction

RNA was prepared from the samples according to RNeasy kit (Qiagen). One μg of RNA was reverse transcribed into cDNA with the use of qScript cDNA Supermix (Quanta Biosciences). Sybr Green (Life Technologies) and primers were used to amplify genes of interest. Gene expression data were normalized to the *GAPDH* or *TBP* genes. Samples were processed in the 7900HT Fast Real-Time Polymerase Chain Reaction (PCR) System, and data were collected with the use of SDS 2.3 software from ABI. Primer sequences used were: *SGK1* forward 5′-CAGCATACGCCGAGCCGGTC-3′, reverse 5′-ATGAAAGC GATGAGAATTGCCACCA-3′; *GAPDH* forward 5′-GAAGGT GAAGGTCGGAGTC-3′, reverse 5′-GAAGATGGTGATGGGA TTTC-3′.

siRNA Knockdown

Reverse transfections were used for all E-Osis and NoEM transfections. Specifically, 40 nmol/L On-Target Plus Non-Targeting siRNA no. 1 (D-001810-01-05; Thermo Scientific), On-Target Plus Human SGK1 (6441) siRNA (J-003027-14-0010; Thermo Scientific), 100 nmol/L Silencer Select siESR2 (s4827; Ambion) or Silencer Select Non-Targeting siRNA no. 1 (4390843; Ambion) were diluted in Opti-MEM Reduced Serum Media with RNAiMAX (Life Technologies) in a 1:5 siRNA:RNAiMAX ratio. Complex formation was carried out for 15 minutes at room temperature and then added to serum-free/antibiotic-free DMEM/F-12 medium overnight. The medium was changed the following day to complete FBS- and antibiotic-containing media. The cells were lysed for mRNA or protein collection 48–96 hours after transfection.

Chromatin Immunoprecipitation

We performed ERβ chromatin immunoprecipitation (ChIP) in E-Osis cells following the protocol published by Lee et al. (27) and using the conditions for ERβ ChIP used by Charn et al. (28). Briefly, cells were grown until 70%–80% confluency

and serum starved overnight, followed by a 45-minute treatment with 100 nmol/L E2. Protein lysates were incubated with 4 μg each of an ERβ antibody mixture (CWK-F12 [28]; PA1–311 [Pierce], GTX70182 [Genetex], or GR-40 [Calbiochem]) or IgG (Sigma) overnight at 4°C. Dynal Beads (Life Technologies) were used to capture the protein/DNA/antibody complexes. After DNA purification, quantitative real-time PCR was performed on INPUT and ChIPed DNA with the use of primers spanning 2.5 kb upstream and downstream of the SGK1 transcription start site. Data were quantified as fold enrichment relative to IgG and normalized to vehicle treatment. Experiments were replicated in E-Osis stromal cells from at least three subjects. Primers used were: SGK1 forward −2.5k b 5′ agcagacatgggccagttac-3′, reverse 5′-gcgagactccgtctcaaaac-3′; SGK1 forward −2 kb 5′-ttgcaacaaagcaaaccaag-3′, reverse 5′-catgtgaaacgccttttcct-3′; SGK1 forward −1.5 kb 5′-atgacctgcagggttttcag-3′, reverse 5′-ccaagaacacgtgaggaggt-3′; SGK1 forward −1 kb 5′-tttcagccttgcttggtttt-3′, reverse 5′-aagatttcctgccccgagt-3′; SGK1 forward −0.5 kb 5′-gagggtatctgcagggacag-3′, reverse 5′-cggggtagttttccacctct-3′; SGK1 forward +1 kb 5′-TCCTCCTTCATCCACAGCTT-3′, reverse 5′-ttcctcaaatccggtcaaac-3′; SGK1 forward +1.5 kb 5′-agtggcgagctggattctaa-3′, reverse 5′-atgcacggcacatacaaaaa-3′; SGK1 forward +2 kb 5′-agCCAAGTCCTTCTCAGCAA-3′, reverse 5′-TTCCAAAACTGCCCTTTCC-3′; SGK1 forward +2.5 kb 5′-ggcggtagacactccttgaa-3′, reverse 5′-CAGGAAAGGGTGCTTCACAT-3′; SGK1 forward ChIP BS 5′-ttggccaaaagcacaaaaa-3′, reverse 5′-gagcactgacgtttccttga-3′.

Immunohistochemistry

We obtained frozen or paraffin-embedded sections of endometriotic tissue from women with pathologically confirmed ovarian endometriosis and of normal endometrium tissue from women without endometriosis. Paraffin-embedded sections were deparaffinized and rehydrated with serial washes in xylene and 100%, 90%, 80%, and 60% ethanol followed by washing in deionized water. Sections underwent antigen retrieval, blocking in 5% normal donkey serum, and incubation with SGK1 (cat. no. ADI-KAP-PK015-D; Enzo) antibody for 1 hour at room temperature in a humidified chamber. Secondary antibody was added for 1 hour at room temperature in the dark, followed by incubation with DAPI (5 ng/mL) in 1X Tris-buffered saline (TBS) and 2 washes in TBS. Tissue sections were placed under coverslips using Prolong Gold antifade mounting media (Life Technologies). Image J was used to calculate the mean intensity of the SGK1 immunofluorescence. Briefly, the SGK1 (red) intensity values were calculated by designating a region of interest (ROI) and plotting the mean signal intensity per outlined area. Results were plotted as mean ± SD.

Immunoblots

Protein from stromal cells was performed with the use of M-PER (Pierce) lysis buffer and quantified with the use of the BCA Protein Assay (Pierce) as indicated by the manufacturer's instructions. At least 20 μg of protein was diluted with reducing 4× LDS Sample Buffer (Life Technologies), electrophoresed on 4%–12% Novex Bis-Tris Polyacrylamide Pre-Cast gels (Life Technologies), and transferred onto polyvinylidene difluoride or nitrocellulose membranes. The membranes were blocked with the use of 5% milk in Tris buffered saline with Tween (TBST) and probed for each specific antibody with shaking overnight at 4°C. Horseradish peroxidase–conjugated secondary antibodies (Cell Signaling) were diluted in 5% milk at 1:5,000 and incubated for 1 hour with shaking at room temperature. The membranes were then washed four times in TBST and once in TBS for 10 minutes, followed each time by incubation with chemiluminscence reagent for 5 minutes (Femto [Pierce] or Luminata Crescendo [Millipore]). Film was used to develop the Western blots in a Konika Minolta developer.

Statistical Analysis

All experiments were performed in tissues from three or more subjects and analyzed by means of Student *t* tests or analysis of variance with Tukey multiple comparison post tests with the use of Graphpad versions 5–6.

RESULTS

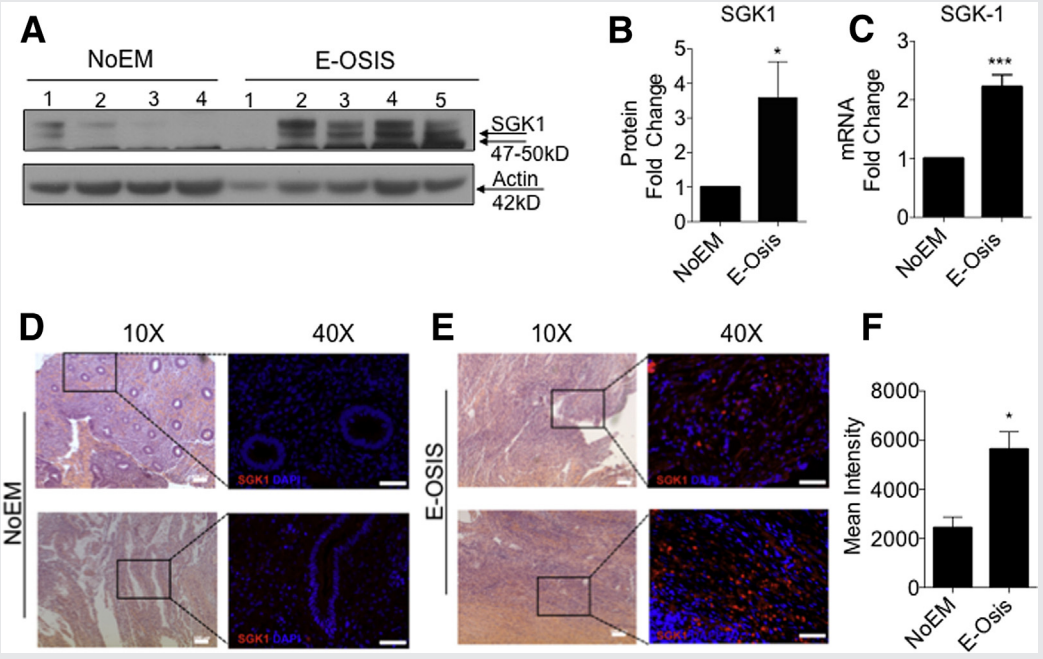
SGK1 is Overexpressed in Endometriosis

We analyzed SGK1 protein levels in stromal cells derived from the endometrium of four normal subjects (NoEM) and from the endometriotic lesions of five subjects with endometriosis (E-Osis; Fig. 1A). Based on densitometric analysis, SGK1 protein levels were 3.59-fold higher ($P<.05$) in E-Osis compared with NoEM cells (Fig. 1B). Quantitative real-time PCR revealed that, compared with NoEM, *SGK1* mRNA levels were 2.1-fold (n = 4; $P<.0005$) higher in E-Osis cells (Fig. 1C). We also performed immunohistochemistry to detect SGK1 in tissue samples of NoEM and E-Osis. As shown in Figure 1D and E, SGK1 protein levels were more abundantly expressed in the diseased tissues compared with the control samples. Quantification of SGK1 immunofluorescence levels confirmed that SGK1 expression was significantly elevated in E-Osis compared with NoEM tissues (mean florescence intensity: NoEM 2,444.5 ± 429.4 vs. E-Osis 5,639.9 ± 696.1; Fig. 1F).

Estradiol Regulates the Expression of SGK1 in Endometriosis

To test whether E2 induces SGK1 expression, we treated E-Osis cells with 10^{-7} mol/L E2 for 2 and 6 hours. We then quantified SGK1 protein expression before and after E2 treatment by means of immunoblot (Fig. 2A). We observed a strong induction of SGK1 protein expression in E-Osis cells following E2 treatment. Although the expression of SGK1 strongly increased after E2 treatment, the levels of SGK1 were not detected under basal conditions, possibly owing to patient-to-patient variation in SGK1 expression. Densitometric analyses demonstrated that E2 increased SGK1 expression 7.49-fold at 2 hours ($P<.05$) and 6.5-fold at 6 hours (not significant; Fig. 2B). To verify whether the E2-mediated induction of SGK1 was due to the transcriptional activation of ERα or ERβ, we treated endometriotic stromal cells with

FIGURE 1



Serum and glucocorticoid–regulated kinase (SGK1) expression is elevated in patients with endometriosis. (**A**) Immunoblot showing that SGK1 protein levels are elevated in stromal cells derived from endometriosis (E-Osis) compared with normal endometrium (NoEM). (**B**) Densitometry analysis showing that SGK1 protein levels are significantly increased in E-Osis compared with NoEM. (**C**) Quantitative real-time polymerase chain reaction was performed on mRNA isolated from NoEM and E-Osis tissues and shows that *SGK1* mRNA is significantly overexpressed in E-Osis compared with NoEM (n = 8). SGK1 immunofluorescence of (**D**) NoEM and (**E**) E-Osis, demonstrating higher expression of SGK1 (*red*) in E-Osis versus NoEM. Nuclei were stained with DAPI (*blue*). Images show hematoxylin and eosin staining imaged at 10× (bar = 100 μm); insets show immunofluorescence imaged at 40× (bar = 40 μm). (**F**) Quantification of the SGK1 immunofluorescence intensity in NoEM and E-Osis (n = 5). *$P<.05$; ***$P<.0001$ (Student *t* test) (48).

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agonists selective for ERα (PPT) or ERβ (DPN). We observed that compared with E2 and PPT, DPN more strongly induced the protein expression of SGK1 (Fig. 2C); however, densitometric analyses showed that the differences induced by E2, PPT, and DPN were not significantly different (Fig. 2D). These results indicated that in E-Osis, E2 induces the protein expression of SGK1.

SGK1 is Transcriptionally Regulated by ERβ

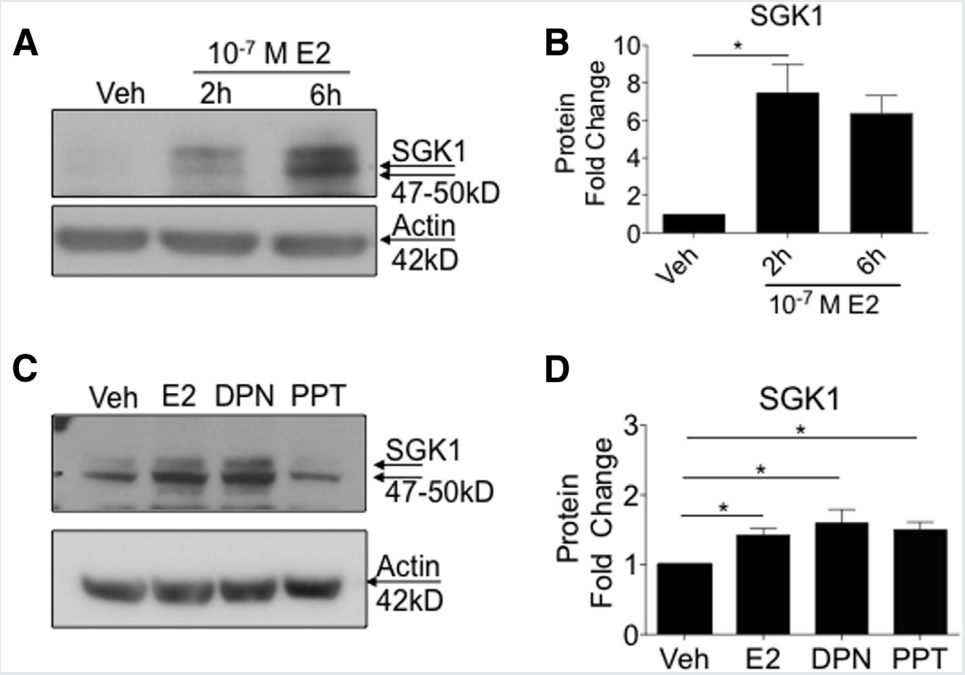
We performed ChIP of ERβ followed by quantitative PCR to confirm that ERβ was enriched at the SGK1 promoter. Previous studies showed that ERβ is frequently recruited to DNA regions rich in SP1 consensus sites (29). Transcription factor binding analysis indicated that several half EREs and SP1 sites were located in the region 2.5 kb upstream of the SGK1 transcription start site (Fig. 3A). We performed ERβ ChIP in E-Osis cells treated with vehicle or 10^{-7} mol/L E2 for 45 minutes. We then amplified the promoter region of SGK1 up to −2.5 kb upstream of the transcription start site. The *SGK1* promoter showed significant ERβ enrichment after E2 treatment at two of the sites analyzed: −2kb (3.59-fold; $P<.001$) and −2.5kb (2.60-fold; $P<.05$) upstream of the *SGK1* transcription start site (Fig. 3A). This confirmed that ERβ was enriched at the SGK1 promoter.

To further demonstrate that ERβ transcriptionally regulates *SGK1*, we performed siRNA knockdown of ERβ in E-Osis cells. As shown in Figure 3B, we observed decreased SGK1 expression after ERβ knockdown. Densitometric analyses of the immunoblots were performed in cells isolated from different subjects (n = 3; Fig. 3C and D). These results showed that a significant decrease in SGK1 expression (0.54-fold; $P=.003$) was obtained after ERβ knockdown (Fig. 3D). We concluded that in endometriotic stromal cells, ERβ contributes to the transcriptional regulation of SGK1.

SGK1 Inhibits Apoptotic Processes in E-Osis Stromal Cells

Based on previous studies indicating that SGK1 promotes cell survival (24, 30), we hypothesized that SGK1 contributes to endometriotic stromal cell survival. To test the effects of decreased SGK1 expression in endometriosis, we performed siRNA-mediated knockdown of SGK1 in E-Osis cells. After knockdown of SGK1, we detected increased expression of cleaved PARP, an apoptosis-related protein that is cleaved by caspase 3 (Fig. 4A). Densitometric analysis of E-Osis cells

FIGURE 2



Estradiol regulates serum and glucocorticoid–regulated kinase (SGK1) via estrogen receptor (ER) β in endometriosis. (**A**) Endometriotic stromal cells were treated with 10^{-7} mol/L E2 for 2 hours and 6 hours. Immunoblot shows that SGK1 protein levels increased in response to E2. (**B**) Densitometry analysis (n = 5) shows that SGK1 protein levels increased significantly in response to E2 treatment after 2 hours. (**C**) Endometriotic stromal cells were treated with E2, DPN (ERβ agonist), and PPT (ERα agonist) for 2 hours, followed by SGK1 protein analysis. (**D**) Densitometry analysis (n = 3) demonstrates that E2, DPN, and PPT significantly induce SGK1 protein expression in endometriosis. *P<.05; (2-way analysis of variance with Tukey multiple-comparison post test).

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from four additional subjects (Fig. 4B and C) demonstrated that after SGK1 knockdown (0.22-fold; P=.001) there was a statistically significant increase in cleaved PARP expression (2.22-fold; P=.03). These results indicate that in endometriosis, SGK1 may contribute to increased endometriotic cell survival by inhibiting apoptosis.

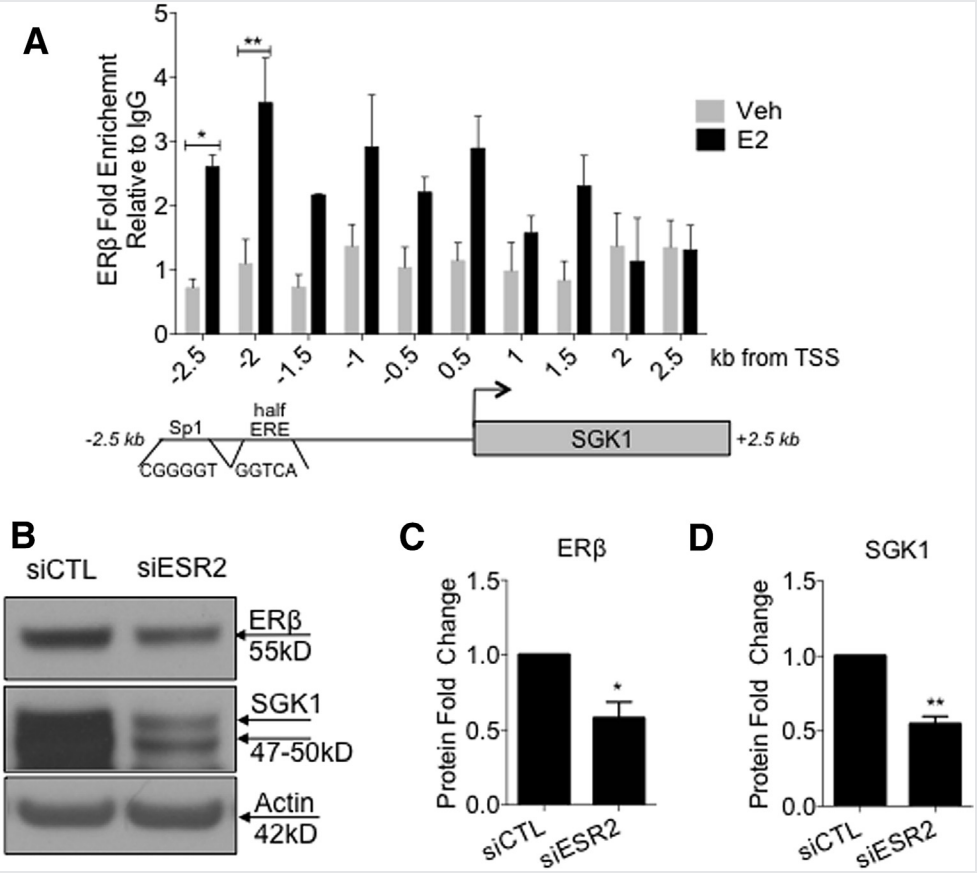
SGK1 is Induced in Response to PGE2

Previous studies showed that oxidative and proinflammatory stress activate SGK1 (24, 31). SGK1 then confers resistance to apoptosis by phosphorylating and inactivating FOXO3a, a transcription factor that induces apoptosis (23, 32). When phosphorylated by SGK1, FOXO3a is excluded from the nucleus, thereby inhibiting its proapoptotic function (24). We hypothesized that SGK1 confers E-Osis survival by phosphorylating FOXO3a, leading to its inactivation and loss of apoptotic function. We treated E-Osis cells with DPN and the proinflammatory factor, PGE2, which induces survival signaling in E-Osis (33–35). In Figure 4D, we show that DPN and PGE2 significantly induced SGK1 expression compared with vehicle-treated cells. Furthermore, PGE2 synergized with DPN to further increase SGK1 in E-Osis. We then measured the phosphorylated and total levels of FOXO3 after DPN and PGE2 treatment. We observed that DPN and PGE2 increased the phosphorylation of FOXO3a, correlating with the increased levels of SGK1. These results indicate that in E-Osis, SGK1 may contribute to cell survival by phosphorylating and inactivating FOXO3a (Fig. 4E).

DISCUSSION

We found that SGK1 is transcriptionally regulated by ERβ, and that it may contribute to increased E-Osis cell survival through inactivation of proapoptotic processes. Previous research showed that ERβ is increased in endometriosis and that signaling via ERβ is important for establishing and maintaining the disease (16, 36, 37). Inflammation is a major contributor to the pain associated with endometriosis, and recent studies demonstrated that inflammation is also involved in the pathogenesis of the disease. For example, ectopic and eutopic endometrium express a proteolytically modified isoform of the steroid receptor coactivator protein (SRC-1) (37). Cleaved SRC-1 renders the ectopic endometrium resistant to the inflammatory and apoptotic signals elicited by tumor necrosis factor. In preclinical studies, compounds that selectively target ERα and ERβ effectively block lesion growth, angiogenesis, and neurogenesis associated with endometriosis (38). Recently, ERβ gain of function was shown to contribute to endometriotic lesion establishment and progression by evading the immune cell surveillance in mice

FIGURE 3



ERβ regulates the transcription of SGK1 in endometriosis. (**A**) Chromatin immunoprecipitation of ERβ followed by quantitative polymerase chain reaction was performed in E-Osis cells (n = 3) treated with 10^{-7} mol/L E2 for 45 minutes. ERβ was significantly enriched ~2kb upstream of the SGK1 transcriptional start site (TSS) after E2 treatment. (**B**) siRNA-mediated ERβ knockdown in E-Osis cells resulted in decreased SGK1 protein expression. (**C** and **D**) Densitometry analysis (n = 3) shows that SGK1 protein levels were significantly decreased in E-Osis after ERβ knockdown. *$P<.05$; **$P<.001$; (Student *t* test or 2-way analysis of variance with Tukey multiple-comparison post test). Abbreviations as in Figure 2.

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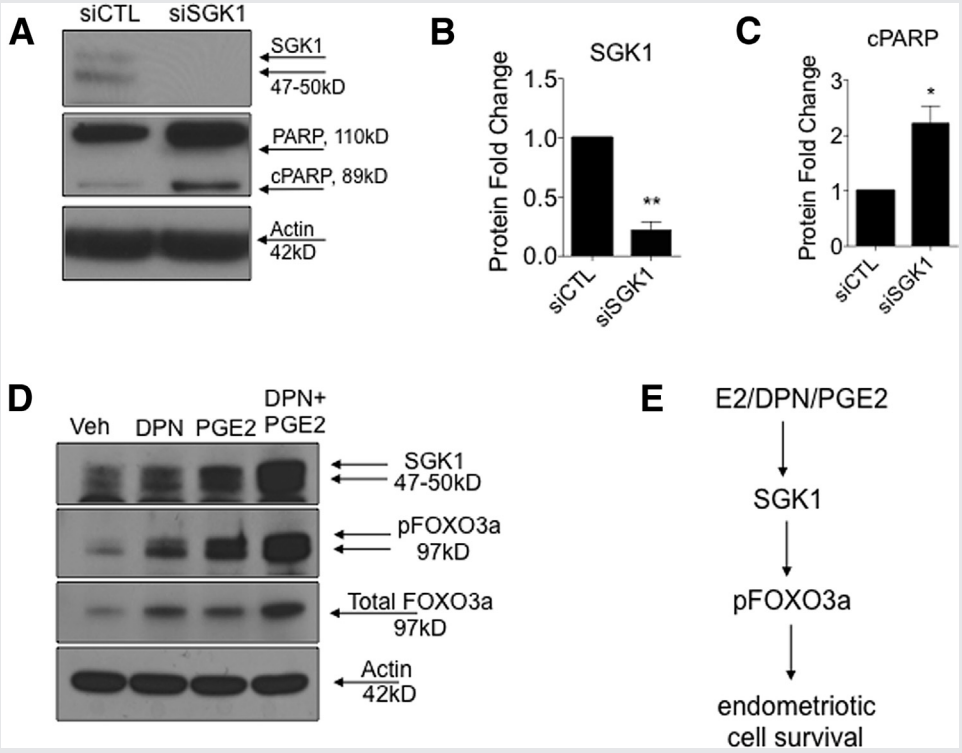
(39). Furthermore, the inflammatory milieu of the disease, which is characterized by elevated PGE2, activates E2 synthesis in ectopic endometriotic stromal cells via SF1 and CYP19A1 activation (25, 34, 40, 41). In the present study, we demonstrated that ERβ and PGE2 activate SGK1, leading to inhibition of proapoptotic factors that may support increased survival of endometriotic stromal cells.

SGK1 is a substrate for various kinases, including BMK1, ERK5, and mammalian target of rapamycin (mTOR) (42–44). When SGK1 is phosphorylated by mTOR, SGK1 then phosphorylates p27, a kinase inhibitor protein (KIP) that phosphorylates and inactivates the cyclin E/cdk2 complexes and inhibits cell cycle progression (44). SGK1 phosphorylation of p27 prevents its nuclear importation and renders it nonfunctional. Thus, SGK1 regulates pathways that ultimately result in cell proliferation. SGK1 also activates kinases that promote cell survival, such as GSK3β and B-Raf (22, 45), and phosphorylates and inactivates the proapoptotic transcription factor FOXO3a. FOXO3a activates the transcription of apoptosis-related genes, such as *TRAIL, IGFBP3,* and *STK11* (24, 32). Studies conducted in human endometrial stromal cells also demonstrate a role for FOXO1/FOXO3a during stromal cell decidualization (46). In those studies, the lack of FOXO3a activation in decidualized endometrial stromal cells confers resistance to the high levels of oxidative stress exerted during placental trophoblast invasion. Thus, in the absence of FOXO3a, decidualized endometrial cells evade apoptosis induced by elevated free radicals (46). Thus the effects of SGK1 on cell proliferation and resistance to apoptosis indicate its importance in cell survival.

Aberrant regulation of SGK1 in the endometrium is associated with infertility or with recurrent pregnancy loss, depending on the expression level (30, 31). SGK1 is highly up-regulated in the endometrium of women with unexplained infertility (31). It is highly up-regulated in response to cyclic adenosine monophosphate and P and is necessary for endometrial stromal cell decidualization (30). Implantation failure occurs in mice with ectopic expression of constitutively active SGK1 (S422D) during the window of implantation. Likewise,

FIGURE 4



SGK1 inhibits apoptosis in endometriotic stromal cells. (**A**) Small interfering (si) RNA knockdown of SGK1 increases cleaved poly(ADP-ribose) polymerase (PARP) expression. Densitometry analysis (n = 4) showing (**B**) significant decrease in SGK1 expression and (**C**) increase in cleaved PARP expression after SGK1 knockdown compared with control samples (siCTL). (**D**) DPN and prostaglandin E2 (PGE2) treatments induce SGK1 expression and FOXO3a phosphorylation in E-Osis stromal cells. (**E**) Diagram showing SGK1 activation in response to E2/DPN/PGE2 and its downstream effects on cell survival via phosphorylation and inactivation of FOXO3a. Immunoblots are representative of experiments run with NoEM and E-Osis cell samples from three study subjects. *$P<.05$; **$P<.001$; (Student *t* test). Abbreviations as in Figures 1 and 2.

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Sgk1$^{-/-}$ mice experience spontaneous pregnancy loss despite having normal implantation rates. In human endometrial stromal cells, SGK1 confers resistance to oxidative stress during in vitro decidualization. These results indicate that SGK1 expression is finely regulated in the uterus to confer normal reproductive function. Here we show that in endometriosis, elevated expression of SGK1 contributes to the survival of the endometriotic stromal cells.

Our results suggest that the local microenvironment of the endometriotic lesion may affect the expression levels of SGK1. For example, Eyster et al. (47) demonstrated that compared with normal endometrium, the expression of *SGK1* was elevated in both endometriomas and peritoneal lesions. However, the expression of *SGK1* was higher in endometriomas than in the peritoneal lesions. As we showed in our studies, SGK1 expression is sensitive to E2 and PGE2, suggesting that the factors present in the microenvironment of the lesion may account for the differences in expression.

In this study, we present data that demonstrate another mechanism by which ERβ signaling contributes to the pathology of endometriosis. We show that ERβ transcriptionally regulates the expression SGK1, a kinase that supports endometriotic stromal cell survival through inhibition of proapoptotic pathways. SGK1 contributes to cell survival through the phosphorylation and inactivation of FOXO3a. The ERβ agonist DPN and PGE2 synergize to activate SGK1 expression. Future studies testing the efficacy of SGK1 inhibitors for the treatment of endometriosis are needed.

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