

Stretch magnitude- and frequency-dependent cyclooxygenase 2 and prostaglandin E2 up-regulation in human endometrial stromal cells: possible implications in endometriosis

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Abstract

Endometriosis, with a prevalence rate ranging from 6% to 10%, is the major contributor to pelvic pain and subfertility, and considerably reduces the quality of life in affected women. However, the pathogenesis of this disease remains largely unknown. The present study aimed to uncover the role of hyperperistalsis in the pathogenesis of endometriosis, by exploring the response of human endometrial stromal cells (ESCs) to the cyclic stretch *in vitro*. ESCs isolated from 18 different endometrium biopsies undergoing hysterectomy for myoma were subjected to uniaxial cyclic stretches with different magnitude and frequency using the Uniaxial Tension System. Expression of cyclooxygenase-2 (COX-2) and microsomal prostaglandin E2 synthase-1 (mPGES-1) in stretched and unstretched ESCs were assessed by realtime quantitative polymerase chain reaction and Western blot. Production of prostaglandin E2 (PGE₂) in the culture medium was measured by enzyme-linked immunosorbent assay. The cyclic stretch mimicking hyperperistalsis in endometriosis (5% elongation at 4 cycles/min) stimulated quick up-regulations of COX-2 and mPGES-1 simultaneously on both transcriptional and translational levels, and delayed PGE₂ overproduction was also noted in ESCs. As the stretch magnitude or frequency increased, so did overexpression of COX-2 and PGE₂ ($P < 0.05$). By contrast, the cyclic stretch mimicking physiological peristalsis (3% elongation at 2 cycles/min) did not induce significant COX-2, mPGES-1 or PGE₂ production within 12 h. Both COX-2 and mPGES-1 are PGE₂ synthases, and the aberrant COX-2 and PGE₂ production play important roles in the pathogenesis of endometriosis. Therefore, the present findings revealed that increased stretch stimuli from the hyperperistalsis of endometriosis were capable of causing the aberrant COX-2 and PGE₂ expression in the endometrium by mechanotransduction, in a magnitude and frequency-dependent manner. It implied possible roles of hyperperistalsis in the pathogenesis of endometriosis, particularly in the view of COX-2 and PGE₂.

Keywords: endometriosis, mechanical stretch, COX-2, PGE₂, human endometrial stromal cell

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Introduction

Endometriosis, defined as endometrial-like tissue outside the uterus, is a major contributor to pelvic pain and subfertility. Affecting 6–10% of women of reproductive age,¹ it considerably reduces the quality of life in affected women to a great extent.² However, most patients of endometriosis receive symptomatic treatments with a rather high recurrence rates,³ because of the unclear pathogenesis.

Numerous theories have been proposed to elaborate the pathogenesis of endometriosis, but none of them have

produced a complete explanation.^{4–9} Among these theories, the role of the eutopic endometrium has recently gained more attentions.¹⁰ Based on the aberrant eutopic endometrium theory, some researchers have suggested that the subendometrial myometrium might also be involved,^{11,12} because the subendometrial myometrium and the endometrium are not only anatomically consecutive, but also of identical origin and are functionally associated.^{13–15} On account of the fact that hyperperistalsis and dysperistalsis of the subendometrial myometrium have presented in endometriosis patients throughout the menstrual cycle,^{16,17} it is

apparent that the endometrial cells of the patients suffer more mechanical stretch stimuli than that of healthy women. However, whether and how the hyperperistalsis or increased mechanical stimulus is involved in the pathogenesis of endometriosis is still the fundamental issue, and to our knowledge, little evidence is yet available.

Given that cells can transduce mechanical signals into a cascade of biochemical signals,¹⁸ we hypothesized that the hyperperistalsis of subendometrial myometrium might lead to the dysfunctional eutopic endometrium by mechanotransduction and contribute to the pathogenesis of endometriosis. The present study investigated the effect of uniaxial cyclic stretch on human endometrial stromal cells (ESCs), using an *in vitro* model mimicking physiological peristalsis (healthy women) and hyperperistalsis (endometriosis patients). In addition, we determined whether this mechanical stretch-mediated response was magnitude- or frequency-dependent. Cyclooxygenase-2 (COX-2) and microsomal prostaglandin E2 synthase-1 (mPGES-1) and prostaglandin E2 (PGE₂) were chosen as indicators to test the hypothesis, because all of them have been reported to play crucial roles in the pathogenesis of endometriosis.^{19–22} COX-2 and mPGES-1 are PGE₂ synthases, which can promote the production of PGE₂.²³ Particularly, dysfunctional expression of COX-2 in the eutopic endometrium of endometriosis has been widely reported.^{20,24,25} By demonstrating the role of hyperperistalsis in the pathogenesis of endometriosis, the present study may broaden the understanding of the disease.

Materials and methods

Patients and endometrial tissue samples

The study was approved by the Ethics Committee of Peking Union Medical College Hospital (PUMCH), and signed informed consent was obtained from each woman. Eighteen women of reproductive age (20–45 y old) undergoing hysterectomy at the Department of Obstetrics and Gynecology in PUMCH between 1 October 2010 and 31 May 2012 were recruited. Considering that dysfunctional subendometrial peristalsis was more noticeable in the secretory phase of endometriosis,²⁶ we only included patients confirmed to be in the pathological secretory phase. Eligibility criteria required individuals to be diagnosed as intramural or subserous myoma (submucous myoma was excluded) by excluding endometriosis or adenomyosis with laparoscopy and pathology. Patients diagnosed with endometrial disease, such as endometrial carcinoma (or atypical hyperplasia), endometrial polyps and others, were excluded. None had received an intrauterine device, hormone therapy or gonadotropin-releasing hormone analog treatment within three months before the surgery. The endometrial tissue was collected under sterile condition during the surgery. Then, it was placed in a sterile tube containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin and 2.5 µg/mL amphotericin B, and transported on ice to a laboratory within eight hours for the isolation and primary culture of ESCs.

Isolation and culture of ESCs

ESCs were isolated and cultured using the protocol reported by Ryan *et al.*²⁷ with minor modifications. Briefly, after rinsing in phosphate-buffered saline (PBS), endometrial tissue was minced into small pieces and digested in DMEM containing type III collagenase (1 mg/mL, 200 U/mg; Worthington Biochemical Corporation, Lakewood, NJ, USA) and deoxyribonuclease I (0.1 mg/mL, 2000 U/mg; Worthington Biochemical Corporation) for 60 min at 37°C with agitation. The dispersed ESCs were separated through a 40-µm sterile cell strainer, and then harvested in a 100-mm culture plate with DMEM containing 10% fetal bovine serum (FBS), 4 mmol/L L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin and 2.5 µg/mL amphotericin B in humidified 5% CO₂/95% air environment at 37°C. The medium was changed every other day, and cells were passaged following treatment with 0.05% Trypsin-ethylenediaminetetraacetic acid solution. All the ESCs used were between passage 1 and 3 in the study. All the reagents used in the isolation and culture were purchased from Invitrogen Corporation (Grand Island, NY, USA).

Application of cyclic tensile strain using the Uniaxial Tension System

ESCs cultured on compliant membranes were subject to cyclic uniaxial tensile strain by the custom-built Uniaxial Tension System (patent number: 200810111785.4, China), which modified the stretch machine reported by Kurpinski *et al.*²⁸ Beforehand, ESCs were harvested in a sterile silicone membrane (11.3 cm × 2.1 cm × 0.01 cm; Specialty Manufacturing Inc., Saginaw, MI, USA) coated with type I collagen (Sigma, St Louis, MO, USA) at a plating density of 2×10^5 cells per membrane. Cell-seeded membranes were cultured in DMEM with 10% FBS until 90% confluence and changed to serum-free medium at 12 h before application of cyclic strain. Afterwards, the membranes were clamped into grippers that were entirely placed inside a tank filled with serum-free culture medium. The mobile gripper was connected with a stepper motor, which was precisely manipulated by the computer. Therefore, the membranes were cyclic-stretched at an even pace with defined magnitude and frequency. ESCs were treated with four degrees of magnitude (3%, 5%, 8% and 10%) and frequency (2, 4, 6 and 8 cycles/min) to model the physiological peristalsis and hyperperistalsis (endometriosis) of the subendometrial myometrium according to the previous studies.^{16,17,29–31} Controls for all the stretch experiments consisted of ESCs seeded on membranes and treated identically, but not subject to cyclic stretch. All the experiments were carried out in a temperature-controlled incubator with humidified 5% CO₂/95% air at 37°C.

Cell viability

Before and after the stretch, ESC viability was assessed by trypan blue exclusion test. In brief, 200 µL of 0.4% trypan blue (Sigma) was added to the membrane. The percentage of viable ESCs (cells with unstained nuclei) was calculated

by counting 100 cells in five random fields of view using a light microscope (TH4-200; Olympus, Tokyo, Japan).

RNA extraction, reverse transcription and realtime quantitative polymerase chain reaction

Total RNA was extracted from each membrane at the indicated hour using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quality and quantity of RNA was identified by NanoDrop 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). Aliquots (600 ng) of RNA were used for cDNA synthesis using the SuperScript® III First-Strand Synthesis system (Invitrogen). The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), COX-2 and mPGES-1 were determined by realtime quantitative polymerase chain reaction (PCR) using Applied Biosystems (Foster City, CA, USA) according to the manufacturer's instructions and a previous study.³² In detail, the probes and primers were used as follows: TaqMan assay on-demand gene expression primer/probe sets for COX-2 (assay ID Hs00153133_m1), mPGES-1 (assay ID Hs00610420_m1) and the primer/probe set for GAPDH (human GAPDH endogenous control [VIC/TAMRA probe, Primer Limited; Applied Biosystems]). Realtime quantitative PCR was performed under the following conditions: 95°C for 20 s, 40 cycles of 95°C for three seconds, and 60°C for 30 s. Cycle threshold (Ct) values and the results were determined by ABI Sequence Detection System (Software SDS version 1.3.1; Applied Biosystems). All mRNA Ct values for each sample were normalized by the Ct value of GAPDH in the same sample as follows: $\Delta\text{Ct} (\text{sample}) = \text{Ct} (\text{sample}) - \text{Ct} (\text{GAPDH})$. The relative mRNA level was expressed as the value of $2^{-\Delta\text{Ct}}$ (sample).

Western blot analysis

ESCs on the membrane were lysed with 100 μL cell lysis buffer containing 0.1 mmol/L phenylmethyl sulfonylfluoride (Beyotime Institute Biotechnology, Haimen, Jiangsu, China) and stored at -70°C until further analysis. After concentration testing by a BCA protein assay kit (Pierce, Thermo Fisher Scientific, Rockford, IL, USA), 30 μg total protein for each sample was subject to sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 12% Tris-HCl linear gradient gel with subsequent electroblotting transfer onto poly(vinylidene difluoride) membrane (Millipore, Bedford, MA, USA). The membrane was then blocked with 5% bovine serum albumin for two hours at room temperature, and hybridized overnight with the rabbit anti-human COX-2 (dilution 1:1000; Cell Signaling, Beverly, MA, USA), rabbit anti-human mPGES-1 (dilution 1:250; Cayman Chemical, Ann Arbor, MI, USA) and rabbit anti-human GAPDH (dilution 1:500; Goodhere Biotechnology Corporation, Hangzhou, Zhejiang, China) antibody. It was washed in phosphate-buffered saline with 0.2% Tween 20 before and after incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (dilution 1:5000; Zhongshan Goldenbridge Biotechnology, Beijing, China), and chemiluminescence was detected with Super

Enhanced chemiluminescence detection reagent (Applygen Technologies Inc., Beijing, China) according to the manufacturer's instructions. The density of the bands was analyzed using Quantity One software, version 4.6 (Bio-Rad, Hercules, CA, USA) and all immunoblot data shown are representatives of independent experiments.

Enzyme-linked immunosorbent assay

The concentration of PGE₂ in the culture media collected from unstretched and stretched ESCs was measured using a PGE₂ enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

Parametric data, as determined by the Shapiro-Wilk test, were analyzed using analysis of variance followed by Tukey's test or Dunnett's test for multiple comparisons appropriately with the software package PASW Statistics, version 18.0 (IBM SPSS, Chicago, IL, USA). Spearman correlation was used to reveal the trend when needed. $P < 0.05$ was considered to be statistically significant.

Results

Cell viability and baseline expression of COX-2, mPGES-1 and PGE₂

ESCs isolated from the endometrial tissue remained viable (>90%) throughout the culture period and without change in viability in the following application of cyclic stretch. Low but detectable COX-2, mPGES-1 and PGE₂ (<100 pg/mL) baseline expression were determined by Western blot or ELISA method. Though the baseline expressions fluctuate among women, there was no significant difference on the baseline expression of COX-2, mPGES-1 and PGE₂ between passage 1 and 3 in ESCs from the same patient (data not shown).

Uniaxial cyclic stretch mimicking the hyperperistalsis in endometriosis induced COX-2, mPGES-1 and PGE₂ overexpression in ESCs

At first, we conducted time-course experiments to determine the effect of uniaxial cyclic stretch on COX-2, mPGES-1 and PGE₂ expression in ESCs. The cyclic stretch of 3% elongation at 2 cycles/min and 5% elongation at 4 cycles/min were chosen to imitate the physiological peristalsis and hyperperistalsis in endometriosis, respectively, according to the previous publications.^{16,30,31,33,34}

Figure 1 illustrates the stretch-induced COX-2, mPGES-1 and PGE₂ expression in ESCs. Applications of cyclic stretch mimicking physiological peristalsis did not induce significant COX-2 expression or PGE₂ production within 12 h (Figure 1). However, cyclic stretch mimicking hyperperistalsis in endometriosis stimulated quick up-regulations of gene expression of COX-2 and mPGES-1 simultaneously (Figure 1a and b). Delayed PGE₂ overproduction was also

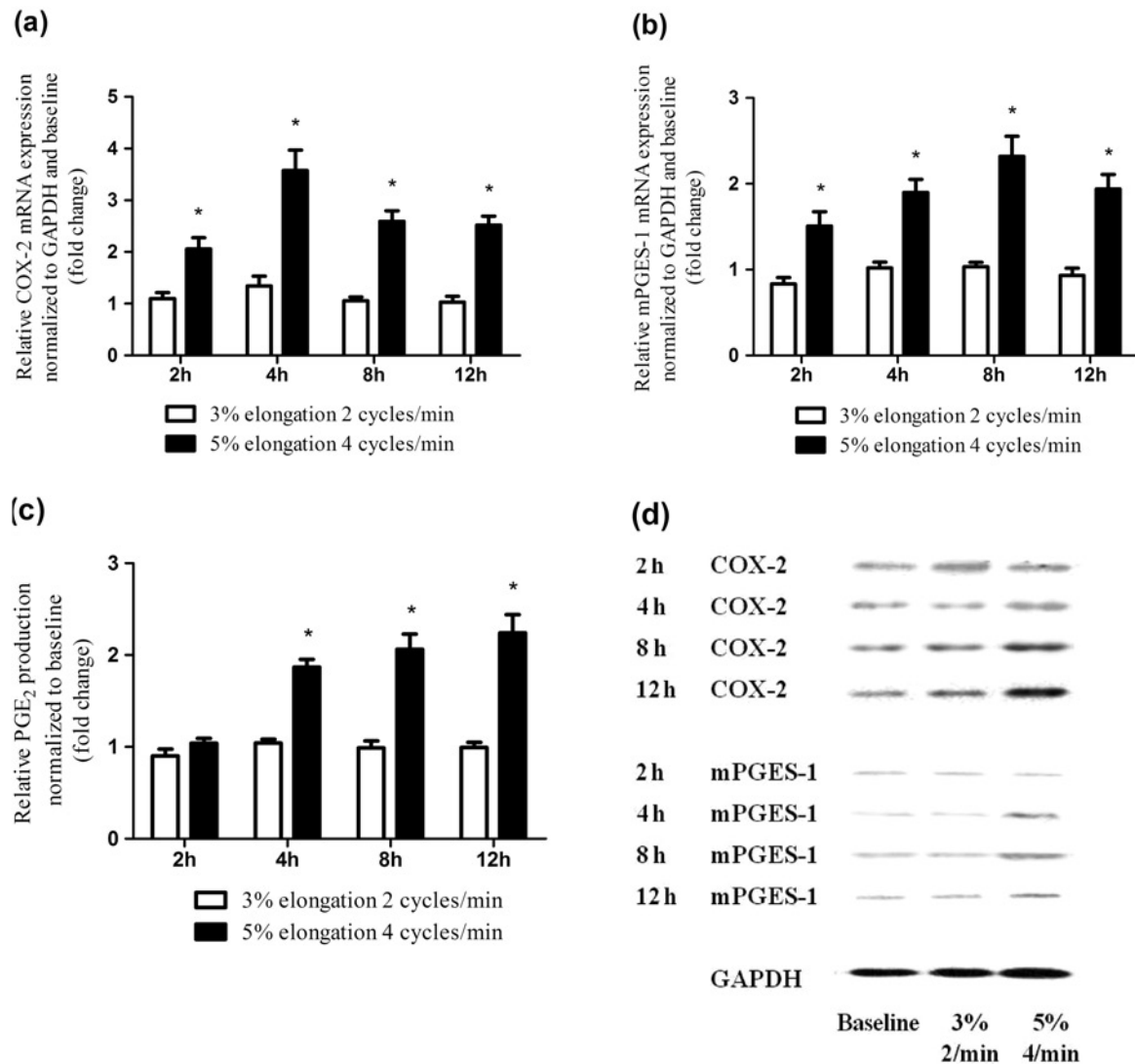


Figure 1 Effect of the uniaxial cyclic stretch on COX-2, mPGES-1 and PGE₂ expression in ESCs. ESCs were mechanically unstimulated or stimulated with cyclic stretch of 3% elongation at 2 cycles/min (white) and 5% elongation at 4 cycles/min (black), and then harvested immediately for the indicated hours. Gene expression of COX-2 and mPGES-1 were determined relative to GAPDH and normalized to the unstimulated control by realtime quantitative PCR (a, b). The production of PGE₂ was assessed by ELISA (c). Typical examples of COX-2 and mPGES-1 protein production were determined by Western blot (d). Values are the mean $\pm$ SEM of samples from four subjects per group. *Statistically significant difference versus unstimulated control. COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mPGES-1, microsomal prostaglandin E2 synthase-1; PGE₂, prostaglandin E2; ESC, human endometrial stromal cell; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay

found, responding to the cyclic stretch mimicking hyperperistalsis in endometriosis (Figure 1c). Under the cyclic stretch of 5% elongation at 4 cycles/min, the expression of COX-2 mRNA came to the peak at four hours (3.67 ± 0.79 fold, $P < 0.05$) and the expression of mPGES-1 mRNA came to the peak at eight hours (2.32 ± 0.47 fold, $P < 0.05$). They smoothly decreased to the platform about two-fold over control at 12 h (Figure 1a and b). The protein level of COX-2 and mPGES-1 production was in line with the mRNA expression. Representative results are shown in Figure 1d. Significant increased PGE₂ production was noted at four hours, and increased steadily as the stretch time increased within 12 h.

After the treatment with cyclic stretch of 5% elongation at 4 cycles/min for four hours, inducible COX-2, mPGES-1 and PGE₂ expression were observed (Figure 2). Interestingly,

overexpressed COX-2 and mPGES-1 expression persisted within the first few hours at the mRNA level (Figure 2a and b). Persistent PGE₂ production was also present within the first four hours (2.28 ± 0.42 fold at four hours, $P < 0.05$) and then decreased to the baseline level gently at 24 h (Figure 2c).

Up-regulation of COX-2, mPGES-1 and PGE₂ by the uniaxial cyclic stretch were frequency-dependent in ESCs

Second, we investigated whether the response to cyclic stretch was frequency-dependent in ESCs. Cyclic stretches of 5% elongation with a gradient frequency from 2 cycles/min to 8 cycles/min were performed on ESCs for four hours. Dynamic COX-2, mPGES-1 and PGE₂ expression as a function

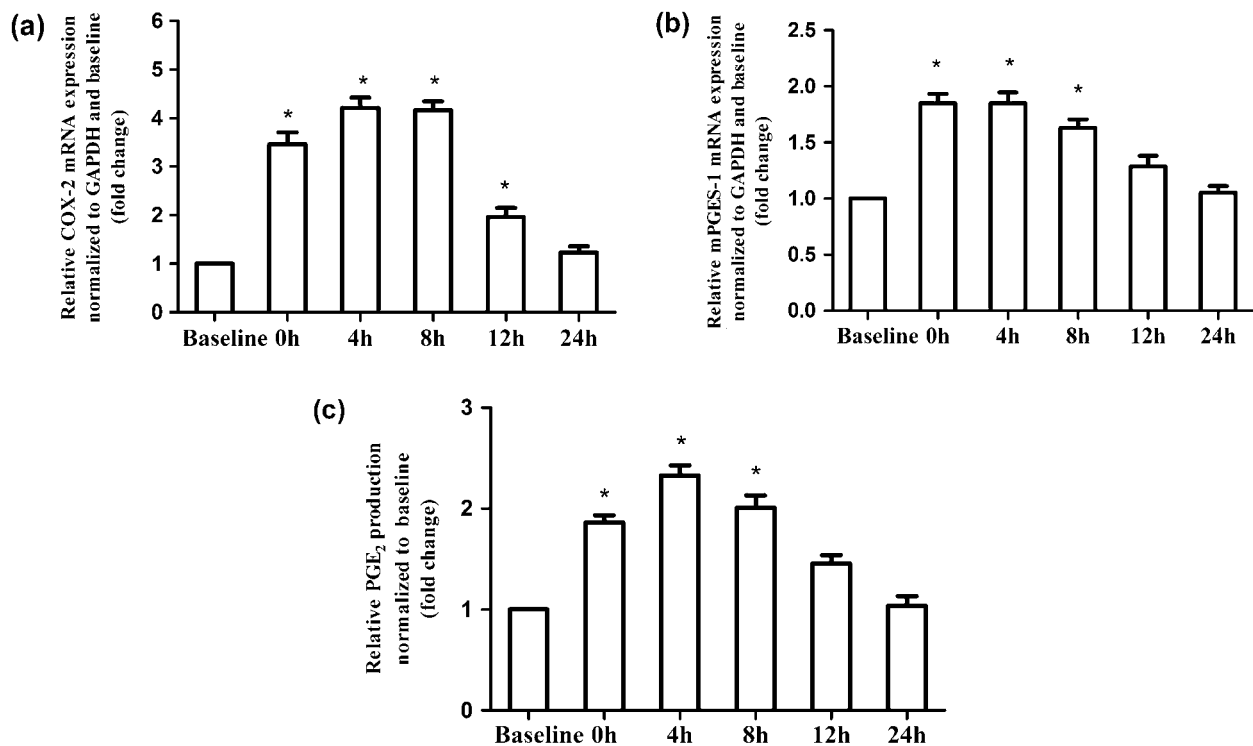


Figure 2 Recovery of COX-2, mPGES-1 and PGE₂ expression in ESCs after cyclic stretch stimuli. After being subjected to cyclic stretch with 5% elongation at 4 cycles/min for four hours, ESCs were immediately harvested or continued to be cultured for indicated hours. Gene expression of COX-2 and mPGES-1 were determined relative to GAPDH and normalized to the unstimulated control by realtime quantitative PCR (a, b). The production of PGE₂ was assessed by ELISA (c). Values are the mean $\pm$ SEM of samples from four subjects per group. *Statistically significant difference versus unstimulated baseline control. COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mPGES-1, microsomal postaglandin E2 synthase-1; PGE₂, prostaglandin E2; ESC, human endometrial stromal cell; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay

of frequency are shown in Figure 3. Only at the frequency higher than 4 cycles/min were significant COX-2, mPGES-1 and PGE₂ inductions presented (Figure 3). Furthermore, there was a significant trend that up-regulation of COX-2, mPGES-1 and PGE₂ expression was frequency-dependent (Spearman correlation, $P < 0.05$).

Up-regulation of COX-2, mPGES-1 and PGE₂ by the uniaxial cyclic stretch were magnitude-dependent in ESCs

Besides the observed frequency-dependence to the cyclic stretch, we also examined the effect of stretching magnitude in ESCs (Figure 4). Significant increased COX-2, mPGES-1 and PGE₂ production were noted with increased stretch magnitude from 3% to 10% elongation at the frequency of 4 cycles/min for four hours in ESCs (Spearman correlation, $P < 0.05$). More than eight-fold of COX-2 protein (Figure 4a) expression was noted with 10% elongation stretch.

Discussion

In the present study, we demonstrated that increased stretch stimuli mimicking the hyperperistalsis of endometriosis induced COX-2, mPGES-1 and PGE₂ overexpression in ESCs *in vitro*. In addition, the stretch-induced COX-2 and PGE₂ production were magnitude- and frequency-dependent with persistent effects. By contrast, the stretch

stimuli mimicking the peristalsis of healthy women did not stimulate significant COX-2, mPGES-1 and PGE₂ overexpression. These findings implied that hyperperistalsis in endometriosis is capable of causing dysfunction in the endometrium by mechanotransduction and may be involved in the pathogenesis of endometriosis.

Because it is quite difficult to study the effect of the stretch-mediated cell regulation *in vivo*, we investigated the mechanical stretch response of ESCs using an *in vitro* model. Compared with the previous studies using a Flexcell system (Flexcell International Co., Hillsborough, NC, USA) to provide the equiaxial stretch,^{30,31} we used the uniaxial system with mild modification according to the machine reported by Kurpinski *et al.*,²⁸ which provided uniaxial or anisotropic strain. It provided mechanical stimuli more similar to *in vivo* conditions of subendometrial myometrial peristalsis, and was more sensitive to uncover the magnitude- and frequency-dependent nature of the stretch-mediated cell response.^{28,35}

COX-2 and PGE₂ play important roles in increased proliferation, migration and invasion of the ESCs in endometriosis, and promote favorable implantation of endometrial cells outside the uterus by perpetuating a positive feedback loop that leads to local inflammation and hyperestrogen.^{19–21} COX-2 was widely reported to be overexpressed in the eutopic endometrium of endometriosis patients.^{20,24,25} As a PGE₂ synthase, mPGES-1 is preferentially coupled with the inducible COX-2 to promote PGE₂

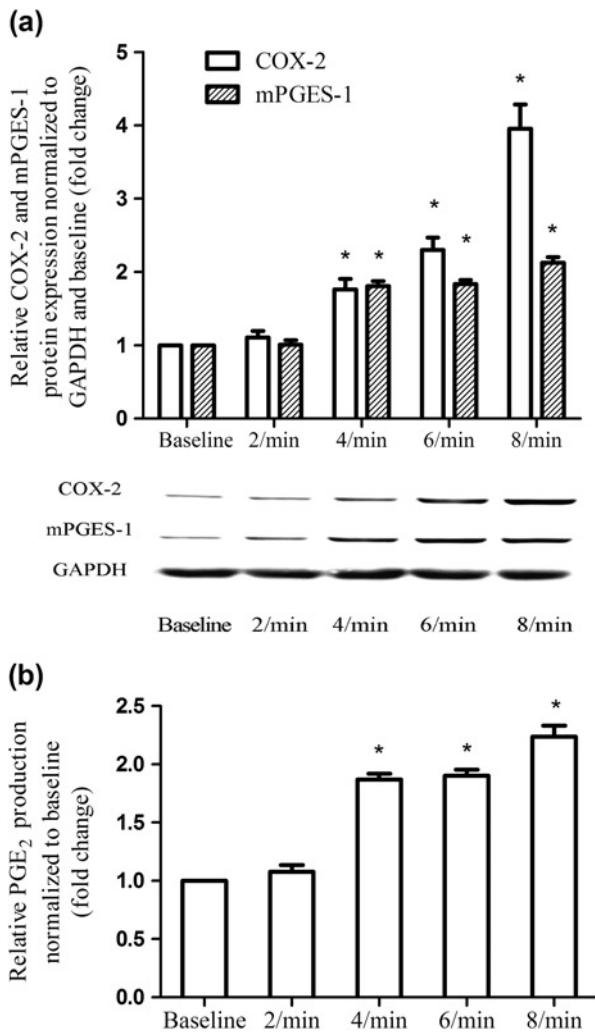


Figure 3 Effect of frequency on COX-2, mPGES-1 and PGE₂ expression in ESCs. ESCs were mechanically unstimulated or stimulated with cyclic stretch of 5% elongation at gratitude frequency (2, 4, 6 and 8 cycles/min) for four hours. Protein production of COX-2 and mPGES-1 were determined relative to GAPDH and normalized to the unstimulated control by Western blot analysis, and subsequently quantified by densitometry using Quantity One software (a). The production of PGE₂ was assessed by ELISA (b). Values are the mean $\pm$ SEM of samples from six subjects per group. *Statistically significant difference versus unstimulated control. COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mPGES-1, microsomal postaglandin E2 synthase-1; PGE₂, prostaglandin E2; ESC, human endometrial stromal cell; ELISA, enzyme-linked immunosorbent assay

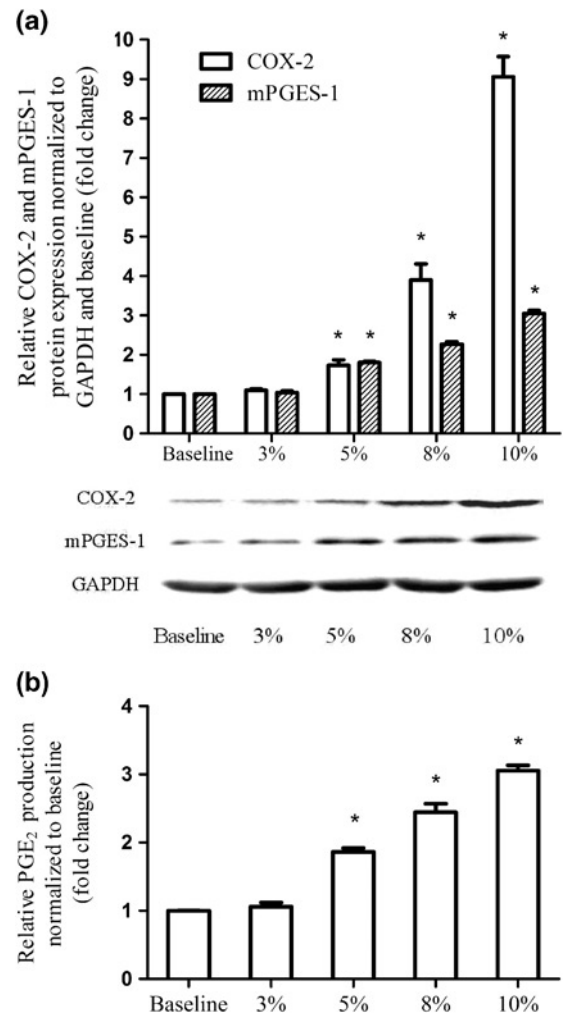


Figure 4 Effect of magnitude on COX-2, mPGES-1 and PGE₂ expression in ESCs. ESCs were mechanically unstimulated or stimulated with cyclic stretch of gratitude magnitude (3% elongation, 5% elongation, 8% elongation and 10% elongation) at 4 cycles/min for four hours. Protein production of COX-2 and mPGES-1 were determined relative to GAPDH and normalized to the unstimulated control by Western blot analysis, and subsequently quantified by densitometry using Quantity One software (a). The production of PGE₂ was assessed by ELISA (b). Values are the mean $\pm$ SEM of samples from six subjects per group. *Statistically significant difference versus unstimulated control. COX-2, cyclooxygenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mPGES-1, microsomal postaglandin E2 synthase-1; PGE₂, prostaglandin E2; ESC, human endometrial stromal cell; ELISA, enzyme-linked immunosorbent assay

generation, and also has been reported to be involved in the pathogenesis of endometriosis.^{22,23} In the present study, quick and persistent overexpression of COX-2, mPGES-1 and PGE₂ were induced by endometriosis-like stretch with a frequency- and magnitude-dependent manner in ESCs, but not by physiological stretch. It indicated that hyperperistalsis of endometriosis may cause the aberrant augmentation of COX-2 and PGE₂ in the endometrium by mechanotransduction. Because COX-2, mPGES-1 and PGE₂ have been reported to be mechanosensitive genes in bladder smooth cells, vascular endothelia, fetal lung epithelia, fibroblasts, cartilage explants and others,³⁶⁻⁴⁰ the findings also corroborated and broadened the previous studies.

Interestingly, after the stretch withdrawal, the gene expression of COX-2 and mPGES-1 did not fall immediately, but continued to be overexpressed in a few hours. Persistent PGE₂ was also noted. It suggested that the cyclic mechanical stretch had a persistent effect on the regulation of COX-2 and PGE₂. With the unusual inherent background and hyperestrogen status in the endometrium of endometriosis *in vivo*,^{19,41} once the ESCs suffered mechanical stimuli which can induce COX-2 or PGE₂ overexpression, a cascade of steroids and inflammatory factors will be subsequently activated, and dysfunction of the endometrium may advance stepwise.

As to the molecular mechanisms by which cells sense and response to the stretch stimuli, we are still largely in the

dark, though several pathways or models have been hypothesized.^{42–46} Zhao *et al.*,³⁷ Mohan *et al.*⁴⁷ and Amma *et al.*³⁹ reported the stretch-induced COX-2/PGE₂ up-regulation mediated by activation of the nuclear factor- κ B pathway in vascular endothelial cells, amnion cells and fibroblasts, respectively. In addition, the Wnt pathway and mitogen-activated protein kinase pathway were also mentioned to produce stretch-induced COX-2/PGE₂ in osteoblastic cells, lung epithelial cells and podocytes.^{48–50} Interestingly, all these pathways were also confirmed to be involved in the dysfunctional eutopic endometrium of endometriosis.^{51–53} This overlapping plausibly implied the possible roles of stretch stimuli in the pathogenesis of the abnormality in eutopic endometrium and endometriosis. As a first step, we did not explore the mechanotransduction pathways involved in the present study. Future studies with delicate design are needed to explore these complicate interactions.

Subendometrial myometrial peristalsis is one of the origins of the uterine movement in non-pregnant women,⁵⁴ which takes part in the process of reproduction, such as the transport of sperm and excretion of shed endometrium.⁵⁵ Some researchers have suggested that hyperperistalsis and dysperistalsis may be involved in the pathogenesis of uterus-related diseases, such as infertility and endometriosis.^{12,30} In previous publications, hyperperistalsis was reported to be involved in the pathogenesis of endometriosis by physical processes, such as facilitating retrograde of the fragments of basal endometrium into the peritoneal cavity^{56,57} and interfering with sperm transportation.⁵⁸ To our knowledge, Harada *et al.*³⁰ reported the phenomena of mechanotransduction in ESCs for the first time in 2005. However, based on the previous studies, the present study revealed the magnitude- and frequency-dependent manner of mechanotransduction in ESCs, which gave an explanation that the hyperperistalsis may induce pathological biochemical signals in the endometrial cells and participate in the pathogenesis of endometriosis subsequently. In addition, from the view of embryonic origin, the subendometrial myometrium is different from the outer myometrium, but the same as the endometrium.^{13–15} It constitutes the archimetra with the endometrium together, which is functionally conservative during development.¹⁷ Our findings also confirmed the link between the subendometrial myometrium and the endometrium by mechanotransduction. It suggested that endometriosis might be an archimetra disease including both the endometrium and the subendometrial myometrium.

Moreover, the hypothesis that the subendometrial myometrium participated in the pathogenesis of endometriosis might provide a potential clinical guide. Previous theory that the abnormal eutopic endometrium plays a decisive role in the pathogenesis of endometriosis promotes studies of endometrial markers for microinvasive diagnosis and direct treatment.^{59–62} Similarly, showing the important role of dysfunctional peristalsis of the subendometrial myometrium in endometriosis might promote non-invasive diagnosis and evaluation in future. Besides, the movements of the subendometrial myometrium can be non-invasively recorded by vaginal sonography and magnetic resonance

imaging.^{17,26,29,63} It could be useful for the diagnosis and follow-up of endometriosis in future, for example using vaginal sonography to evaluate the direction of the subendometrial peristalsis has been reported to be effective in predicting the outcome of *in vitro* fertilization.⁵⁸ More attention should be paid to evaluating the frequency and magnitude of the subendometrial myometrial peristalsis for a better diagnosis, evaluation and follow-up of endometriosis.

Conclusions

Though limited by the *in vitro* nature of the loading culture system, the present study showed that hyperperistalsis was capable of causing the dysfunction of endometrium in a magnitude- and frequency-dependent manner, particular in view of the pathological inducible COX-2 and PGE₂ production. It supported the hypothesis that the subendometrial myometrium took part in the pathogenesis of endometriosis, which broadened the understanding of endometriosis and may provide new methods for diagnosis and evaluation of the disease.

Author contributions: All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript. XL, JL, YF and XG were responsible for the design of the study and data analysis. XL conducted the experiments. YF and XG designed the experiment set-up and conducted part of the experiment. XL, JL and LZ wrote the manuscript. LZ, JL, QF and DS were responsible for patient enrollment and tissue collection.

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