endometrioma by Busacca *et al.* (1999). To analyse risk factors that might influence the recurrence of endometrioma after laparoscopic excision, we retrospectively evaluated 14 variables to assess their independent effects on the recurrence.

Materials and methods

Subjects

A total of 224 patients who had a minimum of 2 years of post-operative follow-up after laparoscopic ovarian endometrioma excision performed at University of Tokyo Hospital between 1995 and 2002 were studied retrospectively. Patient characteristics are summarized in Table I. Institutional Review Board approval was not requested because laparoscopic excision of endometrioma is the standard treatment used in our department. All the procedures followed were in accordance with the revised Declaration of Helsinki, and patients gave informed consent before surgery.

We did not routinely administer pre- or post-operative medical therapy, however, some of the patients were given medical therapy according to their specific needs, e.g. relief of pain. One hundred and two patients had undergone medical treatment previously. Among them, 65 had continued their medication until the operation. The average duration of pre-operative medical therapy was 9.7 months. Post-operative medical therapy was given in 32 cases. The average duration of post-operative medical therapy was 9.5 months. More detailed information about the medication is summarized in Table II.

Surgery

Laparoscopic excision of ovarian endometrioma was performed as follows. After inspection of the pelvis, the ovary was freed from any adhesions. A sharp cortical incision was made, and a cleavage plane was identified. The capsule of the cyst was stripped away from the normal ovarian tissue completely, using bilateral traction and sharp dissection. Other endometriotic peritoneal implants were excised with scissors or coagulated with bipolar electrocoagulation completely, whereas a part of deep endometriosis might be left untreated. Haemostasis was accurately achieved with bipolar electrocoagulation.

The recurrence of ovarian endometrioma was defined as the presence of cysts with a typical aspect detected by transvaginal ultrasonography (Exacoustos *et al.*, 2003) more than 2 cm in diameter within 2 years of surgery. When the cyst was indistinguishable from a transient corpus luteum cyst or an intraovarian haematoma, the diagnosis of recurrence was made only when the cyst had not disappeared after several successive menstrual cycles. Fourteen variables [age, presence of infertility, pain, uterine myoma, adenomyosis, previous medical treatment of endometriosis, previous surgery for ovarian endometriosis, single or multiple cysts, the size of the largest cyst (see abstract) at laparoscopy, unilateral or bilateral involvement, co-existence of deep endometriosis, revised american society for reproductive medicine (ASRM) score, post-operative medical treatment and post-operative pregnancy] were evaluated to assess their effects on the recurrence of ovarian endometrioma. The pain was defined as requiring analgesia at least once a month for dysmenorrhea or chronic pelvic pain. Univariate analysis of the possible risk factors for recurrence followed by a forward step-wise variable selection and logistic regression analysis were performed to eliminate confounding factors. A P value of less than 0.05 was considered statistically significant.

Table I. Characteristics of patients

Factors	Number of cases (%)
Age (years)	32.2 ± 5.4[a]
Infertility	76 (33.9)
Pain	131 (58.5)
Presence of uterine myoma	18 (8.1)
Presence of adenomyosis	60 (26.9)
Previous medical treatment of endometriosis	102 (45.5)
Previous surgery of ovarian endometrioma	30 (13.4)
Multiple cysts	98 (43.8)
Largest cyst diameter (cm)	5.2 ± 1.9[a]
Bilateral involvement	85 (37.9)
Co-existence of deep endometriosis	63 (28.1)
Revised ASRM score	58.1 ± 32.2[a]
Post-operative medical treatment	32 (14.2)
Post-operative pregnancy	39 (17.4)

ASRM, American Society for Reproductive Medicine
[a]Mean ± SD.

Table II. Number of patients who underwent medical treatment before and after the operation

Treatment	Number of patients who underwent medical treatment	
	Before operation	After operation
GnRH agonist	87	15
Danazol	21	5
Oral contraceptives	5	15

Results

The overall rate of recurrence was 30.4% (68/224). Table III presents P values, odds ratio (OR) and 95% confidence interval (95% CI) of univariate and logistic regression analysis.

Using univariate analysis, age, presence of infertility, pain, uterine myoma, adenomyosis, previous surgery for ovarian endometrioma, single or multiple cysts, unilateral or bilateral involvement, co-existence of deep endometriosis and post-operative medical treatment did not significantly influence recurrence. Previous medical treatment of endometriosis, larger diameter of the largest cyst and higher revised ASRM score appeared to be associated with higher recurrence, whereas post-operative pregnancy was associated with lower disease recurrence.

According to a forward step-wise variable selection, five variables (previous medical treatment of endometriosis, the size of the largest cyst at laparoscopy, co-existence of deep endometriosis, revised ASRM score and post-operative pregnancy) were selected for logistic regression analysis. Significant factors that were independently associated with higher recurrence were previous medical treatment of endometriosis [rate of recurrence was 25.5% (29/112) versus 38.2% (39/102) in untreated versus treated patients, respectively, OR = 2.324, 95% CI = 1.232–4.383, $P = 0.0092$] and larger diameter of the largest cyst (OR = 1.182, 95% CI = 1.004–1.391, $P = 0.0442$). Neither co-existence of deep endometriosis nor higher revised ASRM score was significantly associated with recurrence. Post-operative pregnancy was significantly associated with lower recurrence [rate of recurrence was 34.1% (63/185) versus 12.8% (5/39) in no pregnancy versus pregnancy group, respectively, OR = 0.292, 95% CI = 0.028–0.317, $P = 0.0181$].

Table III. Univariate and logistic regression analysis of factors related to the recurrence of ovarian endometrioma

Factors	Univariate analysis	Logistic regression analysis	
	P values	*P* values	Odds ratio (95% confidence interval)
Age (years)	NS		
Infertility	NS		
Pain	NS		
Presence of uterine myoma	NS		
Presence of adenomyosis	NS		
Previous medical treatment of endometriosis	<0.05	<0.01	2.324 (1.232–4.383)
Previous surgery of ovarian endometrioma	NS		
Multiple cysts	NS		
Largest cyst diameter (cm)	<0.05	<0.05	1.182 (1.004–1.391)
Bilateral involvement	NS		
Co-existence of deep endometriosis	NS	NS	0.456 (0.198–1.052)
Revised score	NS	NS	1.010 (1.000–1.021)
Post-operative medical treatment	NS		
Post-operative pregnancy	<0.05	<0.05	0.292 (0.028–0.317)

ASRM, American Society for Reproductive Medicine

Discussion

Many previous studies discussed the recurrence of ovarian endometrioma after laparoscopic excision, in view of requirements of reoperation (Busacca *et al.*, 1999; Saleh and Tulandi, 1999; Abbott *et al.*, 2003) or pain recurrence (Busacca *et al.*, 1999; Abbott *et al.*, 2003). In this study, we focused on the mechanism of ovarian endometrioma recurrence *per se* and used a definition of the recurrence as the presence of cysts more than 2 cm in diameter by ultrasonography, which might be rather objective and cover minimum lesions. Under this definition, we observed a recurrence rate of 30.4%.

The patient's age, presence of infertility and pain did not significantly influence the recurrence. The presence of neither uterine myoma nor adenomyosis was significant. As for the characteristics of endometrioma, single or multiple cysts and unilateral or bilateral ovarian involvement were not significant, whereas patients with larger endometrioma had higher probability of recurrence, which agrees with the finding of earlier studies (Busacca *et al.*, 1999; Saleh and Tulandi, 1999). Because most ovarian endometrioma are associated with extra ovarian endometriosis (Redwine, 1999), we evaluated revised ASRM score and co-existence of deep endometriosis. Revised ASRM score did not independently correlate the recurrence. Co-existence of deep endometriosis did not influence the recurrence either.

A new observation demonstrated in this study was that previous medical treatment of endometriosis was a significant factor that was associated with higher recurrence, whereas previous surgery of ovarian endometrioma was not. The less-favourable prognosis for women who have already had medical treatment may be explained by two possible reasons. The first is that the medication may mask endometriotic lesions and allow them to escape from removal at operations. Because more than half of the women who were categorized into previous medical treatment group had continued their medication until the time of operation, it may be possible that the medication might yield latent lesions that remain and recur after the operation. Our findings may also support the study of Muzii *et al.* (1996), which suggests that pre-operative GnRH agonist treatment does not seem to offer any advantage in terms of surgical performance based on various parameters including recurrence rates.

The second possible reason for negative impact of medical treatment on endometrioma recurrence is that hormonal suppressive therapy may alter some genomic characteristics of endometriotic lesions. As for malignant transformation of endometriosis, it is proposed that hormonal ablative treatments may cause negative selection, suppress the normal, eukaryotic cells more than aneuploid cells bearing chromosomal aberrations and increase the rate of dyskaryotic cells in the endometriotic implants (Blumenfeld, 2004). We suppose that the 'negative selection' may also contribute to the recurrence of disease, making the lesion more active, progressive and prone to recurrence.

Patient with post-operative pregnancy had a much lower rate of recurrence, which indicates that subsequent pregnancy may have a protective effect on endometrioma recurrence. On the contrary, laparoscopic excision of endometrioma is known to improve fertility, when it is done in infertile women (Beretta *et al.*, 1998). Taken together, gynaecologists should optimize the timing of laparoscopy according to the patient's desire for current and future pregnancy.

Our study was in line with previous observations that post-operative medical treatment did not significantly influence disease recurrence (Bianchi *et al.*, 1999; Muzii *et al.*, 2000; Busacca *et al.*, 2001). Three-month GnRH analogue (Busacca *et al.*, 2001) or danazol (Bianchi *et al.*, 1999) therapy after laparoscopy was demonstrated to provide no significant advantage in preventing disease recurrence. Post-operative administration of low-dose cyclic oral contraceptives for 6 months had also no significant effect on the long-term recurrence rate of endometrioma (Muzii *et al.*, 2000). However, the treatment period of these studies, and also ours, was less than 1 year, and there is no information about the effect of longer period of treatment. It is therefore possible that medical treatments longer than 1 year may have an effect to prevent endometrioma recurrence. Further studies, e.g. randomized controlled trials, are needed to determine the effectiveness of these therapies.

In summary, this study demonstrated significant factors that were independently associated with a higher or lower recurrence of endometrioma after laparoscopic excision.

References

Abbott JA, Hawe J, Clayton RD and Garry R (2003) The effects and effectiveness of laparoscopic excision of endometriosis: a prospective study with 2-5 year follow-up. Hum Reprod 18,1922–1927.

Beretta P, Franchi M, Ghezzi F, Busacca M, Zupi E and Bolis P (1998) Randomized clinical trial of two laparoscopic treatments of endometriomas: cystectomy versus drainage and coagulation. Fertil Steril 70,1176–1180.

Bianchi S, Busacca M, Agnoli B, Candiani M, Calia C and Vignali M (1999) Effects of 3 month therapy with danazol after laparoscopic surgery for stage III/IV endometriosis: a randomized study. Hum Reprod 14,1335–1337.

Blumenfeld Z (2004) Hormonal suppressive therapy for endometriosis may not improve patient health. Fertil Steril 81,487–492.

Busacca M, Marana R, Caruana P, Candiani M, Muzii L, Calia C and Bianchi S (1999) Recurrence of ovarian endometrioma after laparoscopic excision. Am J Obstet Gynecol 180,519–523.

Busacca M, Somigliana E, Bianchi S, De Marinis S, Calia C, Candiani M and Vignali M (2001) Post-operative GnRH analogue treatment after conservative surgery for symptomatic endometriosis stage III-IV: a randomized controlled trial. Hum Reprod 16,2399–2402.

Exacoustos C, Zupi E, Carusotti C, Rinaldo D, Marconi D, Lanzi G and Arduini D (2003) Staging of pelvic endometriosis: role of sonographic appearance in determining extension of disease and modulating surgical approach. J Am Assoc Gynecol Laparosc 10,378–382.

Ghezzi F, Beretta P, Franchi M, Parissis M and Bolis P (2001) Recurrence of ovarian endometriosis and anatomical location of the primary lesion. Fertil Steril 75,136–140.

Giudice LC and Kao LC (2004) Endometriosis. Lancet 364,1789–1799.

Jones KD and Sutton CJ (2000) Laparoscopic management of ovarian endometriomas: a critical review of current practice. Curr Opin Obstet Gynecol 12,309–315.

Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, Hummelshoj L, Prentice A and Saridogan E (2005) ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod 20,2698–2704.

Muzii L, Marana R, Caruana P and Mancuso S (1996) The impact of preoperative gonadotropin-releasing hormone agonist treatment on laparoscopic excision of ovarian endometriotic cysts. Fertil Steril 65,1235–1237.

Muzii L, Marana R, Caruana P, Catalano GF, Margutti F and Panici PB (2000) Postoperative administration of monophasic combined oral contraceptives after laparoscopic treatment of ovarian endometriomas: a prospective, randomized trial. Am J Obstet Gynecol 183,588–592.

Nishida M, Watanabe K, Sato N and Ichikawa Y (2000) Malignant transformation of ovarian endometriosis. Gynecol Obstet Invest 50 (Suppl. 1), 18–25.

Redwine DB (1999) Ovarian endometriosis: a marker for more extensive pelvic and intestinal disease. Fertil Steril 72,310–315.

Saleh A and Tulandi T (1999) Reoperation after laparoscopic treatment of ovarian endometriomas by excision and by fenestration. Fertil Steril 72,322–324.

Submitted on November 4, 2005; resubmitted on February 9, 2006, March 26, 2006; accepted on March 30, 2006

0013-7227/06/$15.00/0
Printed in U.S.A.

Endocrinology 147(7):3203–3210
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doi: 10.1210/en.2005-1510

Expression of Adiponectin Receptors and Its Possible Implication in the Human Endometrium

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Adiponectin, a pleiotropic cytokine, exerts its effects via the specific receptors AdipoR1 and AdipoR2. Whereas circulating adiponectin concentrations decrease in women with endometriosis and endometrial cancer, possible effects of adiponectin and the presence of the receptors in the endometrium have not been determined. In this study, we examined the expression of adiponectin receptors AdipoR1 and AdipoR2 in the human endometrium and assessed effects of adiponectin in endometrial cells. Expression of AdipoR1 and AdipoR2 in endometrial tissues was evaluated by real-time quantitative PCR, *in situ* hybridization, and Western blotting. The effects of adiponectin on phosphorylation of AMP-activated protein kinase, a regulator of energy homeostasis, in cultured endometrial stromal cells (ESCs) and epithelial cells (EECs) were studied by Western blotting. The effects of adiponectin on IL-1β-induced secretion of IL-6, IL-8, and monocyte chemoattractant protein 1 from cultured ESCs were determined using specific ELISAs. The expression of AdipoR1 and AdipoR2 was detected in the endometrium. The expression of both genes was increased in the midluteal phase, the period of embryo implantation. *In situ* hybridization revealed that both AdipoR1 and AdipoR2 appeared to be equally expressed in the epithelial cells and in the stromal cells. Adiponectin increased phosphorylation of AMP-activated protein kinase in ESCs and EECs. Adiponectin decreased IL-1β-induced secretion of IL-6, IL-8, and monocyte chemoattractant protein 1 from ESCs. These findings suggest that adiponectin exerts energy-homeostatic and antiinflammatory effects in the endometrium, and these effects might be relevant to pathological and physiological endometrium-related events such as implantation and endometriosis. (*Endocrinology* 147: 3203–3210, 2006)

ADIPONECTIN IS A hormone that structurally belongs to the complement 1q family (1). It is highly expressed in differentiated adipocytes and circulates at high levels in the bloodstream (2–5). Adiponectin levels both in adipose tissue and in circulation are reduced in obesity (2, 3). An increasing body of evidence indicates that adiponectin plays an important role in regulating energy metabolism and insulin sensitivity (3, 6). In addition to its antidiabetic effects, adiponectin has been shown to have pleiotropic activities such as antiinflammatory, antiangiogenic, and antiatherosclerotic effects (7–9).

Two adiponectin receptors (AdipoR1 and AdipoR2) have recently been identified (10). The receptors contain seven-transmembrane domains but are structurally and functionally distinct from G protein-coupled receptors. Activation of these receptors phosphorylates AMP-activated protein kinase (AMPK), a regulator of energy homeostasis of the cell, and stimulates fatty acid oxidation and glucose uptake. In mice, AdipoR1 is abundantly expressed in the skeletal muscle, whereas AdipoR2 is predominant in the liver (10). In humans, although the expression of the receptors has been reported in a few tissues and cells (11–15), the expression in reproductive organs has been poorly understood.

A growing body of evidence indicates that many adipokines, including adiponectin, have biological implications for female fertility (16). Interestingly, we and others have recently demonstrated that serum adiponectin levels are decreased in women with endometriosis (17) and endometrial cancer (18, 19). Given the diverse effects of adiponectin, these findings imply that adiponectin exerts some effects on the endometrium.

With these backgrounds, we surmised that the adiponectin receptors are expressed in the human endometrium and that adiponectin has possible effects therein. To address this thesis, we studied the presence of the adiponectin receptors in endometrial tissues, and adiponectin-induced activation of the receptors was examined by measuring AMPK phosphorylation of endometrial cells. In addition, effects of adiponectin on IL-1β-induced secretion of IL-6, IL-8, and monocyte chemoattractant protein (MCP)-1 from the endometrial cells was determined, considering that these proinflammatory cytokines are important in pathology and physiology of the endometrium (20–22).

Materials and Methods

Reagents and materials

Type I collagenase and antibiotics (a mixture of penicillin, streptomycin, and amphotericin B) were purchased from Sigma Chemical Co. (St. Louis, MO). DMEM/Ham's F12 (F-12) medium was from Life Tech-

First Published Online April 6, 2006

Abbreviations: AICAR, 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; DIG, digoxigenin; EEC, endometrial epithelial cell; ESC, endometrial stromal cell; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MCP, monocyte chemoattractant protein; SSC, standard saline citrate.

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nologies, Inc. (Grand Island, NY). Human recombinant adiponectin was obtained from R&D Systems (Minneapolis, MN) and generated in our laboratory (6). 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) was obtained from Toronto Research Chemicals (Toronto, Canada). Antihuman rabbit antibodies of AMPK-α (no. 2532) and phospho-AMPK-α (no. 2535) were from Cell Signaling (Beverly, MA). Rabbit antibodies for AdipoR1 (ADIPOR12-A) and AdipoR2 (ADIPOR22-A) were purchased from Alpha Diagnostic International (San Antonio, TX). Antirabbit horseradish peroxidase secondary antibody was from Amersham Biosciences (Little Chalfont, UK). Recombinant IL-1β was purchased from Genzyme/Techne (Minneapolis, MN). Charcoal-stripped fetal bovine serum (FBS) was from HyClone (Logan, UT). Deoxyribonuclease I was from Invitrogen (Carlsbad, CA).

Collection of samples

Endometrial tissues were obtained from women undergoing hysterectomy for benign gynecological conditions. In total, 77 women aged 32–45 yr were recruited to the present study. All women had regular menstrual cycles, and none had received hormonal treatment for at least 6 months before surgery. The tissues collected under sterile conditions were processed for primary cell cultures. The phases of the menstrual cycles were determined and classified as early, mid, and late proliferative and secretory phases according to the last and next menstrual period, basal body temperature, ultrasound findings on the endometrium and ovarian follicles, and standard histological criteria by Noyes et al. (23). Subcutaneous fat tissues of the abdomen were obtained from three women during hysterectomy. The tissues for mRNA extraction and Western blot analysis were snap frozen in liquid nitrogen and stored at −80 C. The tissues for *in situ* hybridization were fixed overnight in 10% formalin neutral buffer solution, subsequently dehydrated with a series of ethanol washes, and embedded in paraffin. The tissues for cell culture experiment were provided for additional preparation.

The experimental procedures were approved by the institutional review board of the University of Tokyo, and signed informed consent for use of the sample was obtained from each woman.

Isolation and culture of human endometrial stromal and epithelial cells

The isolation and culture of human endometrial stromal cells (ESCs) and epithelial cells (EECs) were processed as described previously (20, 21, 24). Fresh endometrial biopsy specimens collected in a sterile medium were rinsed to remove blood cells. The tissues were minced into small pieces and incubated in DMEM/F-12, containing 0.25% type I collagenase and 15 U/ml deoxyribonuclease I, for 60 min at 37 C. The resultant dispersed endometrial cells were separated by filtration through a 40-μm nylon cell strainer (Becton Dickinson and Co., Franklin Lakes, NJ). The endometrial epithelial glands that remained intact were retained by the strainer, whereas the dispersed ESCs passed through the strainer into the filtrate.

ESCs in the filtrate were collected by centrifugation and resuspended in phenol-red-free DMEM/F-12 containing 10% charcoal-stripped FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 mg/ml amphotericin B. The ESCs were seeded in a 100-mm culture plate and kept at 37 C in a humidified 5% CO_2/95% air atmosphere. At the first passage, the cells were plated into six- or 48-well culture plates (Becton Dickinson) at a density of 2×10^5 cells/ml. The cells reached confluence in 2 or 3 d and then were used for the experiments.

EECs were collected by backwashing the strainer with DMEM/F-12 containing 10% charcoal-stripped FBS, seeded in a 100-mm plate, and incubated at 37 C for 60 min to allow contaminated ESCs to attach to the plate wall. The nonattached EECs were recovered and cultured in the culture medium at a density of 2×10^5 cells per well into a 12-well culture plate. The cells that reached confluence in 3 or 4 d were used for the experiments.

The purity of both the stromal and epithelial cell preparations was more than 95%, as judged by positive cellular staining for vimentin and cytokeratin, respectively.

Treatment of the cells

When the ESCs and EECs were approaching confluence, the complete media were removed and replaced with fresh media and antibiotics, and the cells were cultured in serum-free media for an additional 12 h. To examine whether AdipoR1 and AdipoR2 functioned in ESCs and EECs, the cells were incubated with adiponectin for 0–60 min or 1 mM AICAR, an experimental tool to activate AMPK, for 1 h. To evaluate the effects of adiponectin on the IL-1β-induced production of IL-6, IL-8, and MCP-1 in ESCs, the cells were incubated with or without 50 μg/ml adiponectin in serum-free media for 24 h and then stimulated with 5 ng/ml IL-1β in serum-free media for 24 h, according to our previous study (22).

RNA extraction, RT, standard PCR, and real-time quantitative PCR of adiponectin, AdipoR1, and AdipoR2

Total RNA was extracted individually from the endometrial tissue, ESCs, and EECs using an RNeasy minikit (QIAGEN, Hilden, Germany). One microgram of total RNA was reverse transcribed in a 20-μl volume using ReverTra Ace-α (TOYOBO, Osaka, Japan). Standard PCR was performed using ReverTra Dash (TOYOBO) according to the manufacturer's instructions. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (TOYOBO) were used to ensure the quality and amounts of RNA. For negative controls, RNA without RT was used. Adiponectin primers (sense, 5'-AACATGCCCATTCGCTTTAC-3'; antisense, 5'-ATTACGCTCTCCTTCCCCAT-3') were chosen to amplify a 288-bp fragment. AdipoR1 primers (sense, 5'-AAACTGGCAACATCTGGACC-3'; antisense, 5'-GCTGTGGGGAGCAGTAGAAG-3') were chosen to amplify a 300-bp fragment. AdipoR2 primers (sense, 5'-ACAGGCAACATTTGGACACA-3'; antisense, 5'-CCAAGGAACAAAACTTCCCA-3') were chosen to amplify a 267-bp fragment. Both AdipoR1 and AdipoR2 primers span introns. PCR conditions for amplifications of adiponectin, AdipoR1, and AdipoR2 were 30 cycles at 98 C for 10 sec, 60 C for 2 sec, and 74 C for 15 sec. PCR products were analyzed by agarose gel electrophoresis with ethidium bromide.

To assess adiponectin, AdipoR1, and AdipoR2 mRNA expression, real-time quantitative PCR and data analysis were performed using LightCycler (Roche Diagnostic GmbH, Mannheim, Germany), according to the manufacturer's instructions. Expression of adiponectin, AdipoR1, and AdipoR2 mRNA was normalized to RNA loading for each sample using GAPDH mRNA, for which expression was substantially constant during the menstrual cycle, as an internal standard. The primers for adiponectin, AdipoR1, AdipoR2, and GAPDH were the same as those used for standard PCR. PCR conditions were as follows: for adiponectin, 40 cycles at 95 C for 15 sec, 65 C for 8 sec, and 72 C for 12 sec; for AdipoR1, 35 cycles at 95 C for 15 sec, 65 C for 8 sec, and 72 C for 12 sec; for AdipoR2, 35 cycles at 95 C for 15 sec, 65 C for 8 sec, and 72 C for 11 sec. All these PCR conditions were followed by melting curve analysis.

Each PCR product was purified with a QIAEX II gel extraction kit (QIAGEN), and their identities were confirmed using an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA).

In situ hybridization

To prepare the digoxigenin (DIG)-labeled RNA probes for adiponectin, AdipoR1, and AdipoR2, the 288-, 300-, and 267-bp fragments of the

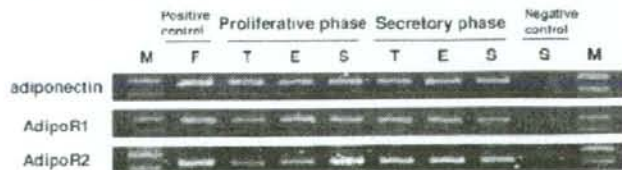

FIG. 1. Expression of adiponectin, AdipoR1, and AdipoR2 mRNA in the endometrium, as detected by standard RT-PCR. Total RNA was extracted from endometrial tissues, cultured EECs, and ESCs in the proliferative phase and the secretory phase. Subcutaneous fat tissues were used for positive controls. For negative controls, RNA without RT was used. Lane T, endometrial tissues; lane E, EECs; lane S, ESCs; lane F, sc fat tissues; lane M, DNA molecular weight standards. The data shown are representative of three different samples in each phase.

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human adiponectin, AdipoR1, and AdipoR2 cDNA, obtained by RT-PCR with the primers described above, were subcloned into the appropriate restriction sites of the PCR II-TOPO vector (Invitrogen). After linearization of plasmid with an appropriate enzyme, the linearized vectors were used as templates for the synthesis of DIG-labeled RNA probes using SP6 or T7 RNA polymerase.

In situ hybridization was performed using an ISHR Starting kit (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. The paraffin-embedded specimens were sliced at a 6-μm thickness. These sections were mounted on poly-L-lysine-treated slides, deparaffinized, and rehydrated. They were further digested with 5 mg/ml proteinase K for 10 min at room temperature, treated with 0.17% acetic anhydrate, and then subjected to treatment with prehybridization solution containing 50% formamide and 2× standard saline citrate (SSC) (1× SSC consists of 0.15 M NaCl and 0.015 M sodium citrate) for 30 min at 42 C. The probe was diluted to a concentration of 0.5 μg/ml in hybridization buffer. Hybridization was carried out by applying the diluted probe (150 μl) to each slide section. Each section was incubated in a humidified chamber overnight at 42 C.

Slides were washed three times in washing solution (50% formamide and 2× SSC) for 20 min each at 42 C, treated with ribonuclease for 30 min at 37 C, and washed three times in 0.1× SSC for 20 min each at 42 C. After being blocked with blocking solution, the sections were incubated with an anti-DIG, alkaline phosphate-conjugated antibody (1:500; Roche) for 60 min at room temperature, and washed three times in washing buffer. Color development was carried out by overlaying them with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate (NBT-BCIP; Roche), and they were incubated in a humidified chamber in the dark for 12 h at room temperature. All sections were evaluated under light microscope. Sense probe hybridization was used as a control for background level.

Western blot analysis

Cultured cells and endometrial tissues were homogenized in the lysis buffer containing 50 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate, 10% glycerol, 50 mM dithiothreitol, and 0.1% bromophenol blue. The lysates were further diluted with lysis buffer to give a final concentration of 1 mg total protein/ml. Samples of 20 μg protein per lane were resolved by 10% (for total AMPK-α and phospho-AMPK-α) and 14% (for AdipoR1 and AdipoR2) SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane and incubated with antirabbit antibodies to AdipoR1 (5 μg/ml) and AdipoR2 (5 μg/ml), total AMPK-α (1:1000), or phospho-specific AMPK-α (1:1000) as primary antibodies and antirabbit horseradish peroxidase as a secondary antibody (1:1000). Immune complexes were visualized by use of the ECL Western blotting system (Amersham Biosciences).

Measurement of IL-8, IL-6, and MCP-1

Concentrations of IL-6, IL-8, and MCP-1 were measured using a specific ELISA kit (Quantikine; R&D Systems) according to the manufacturer's protocol as described previously (22, 25). The sensitivities of the assays were 3.12, 15.6, and 31.2 pg/ml for IL-6, IL-8, and MCP-1, respectively. The intraassay and interassay coefficients of variation were less than 5% in these assays.

Statistical analysis

Data were checked for normal distribution using Bartlett test and evaluated using ANOVA with *post hoc* analysis (Fisher's protected least significance) for multiple comparisons. $P < 0.05$ was accepted as statistically significant.

Results

Expression of adiponectin, AdipoR1, and AdipoR2 mRNA in endometrial tissues, EECs, and ESCs

The expression of adiponectin, AdipoR1, and AdipoR2 mRNA in endometrial tissues, EECs, and ESCs were detected by standard RT-PCR analysis (Fig. 1). A signal of the same size was detected in control sc fat tissues.

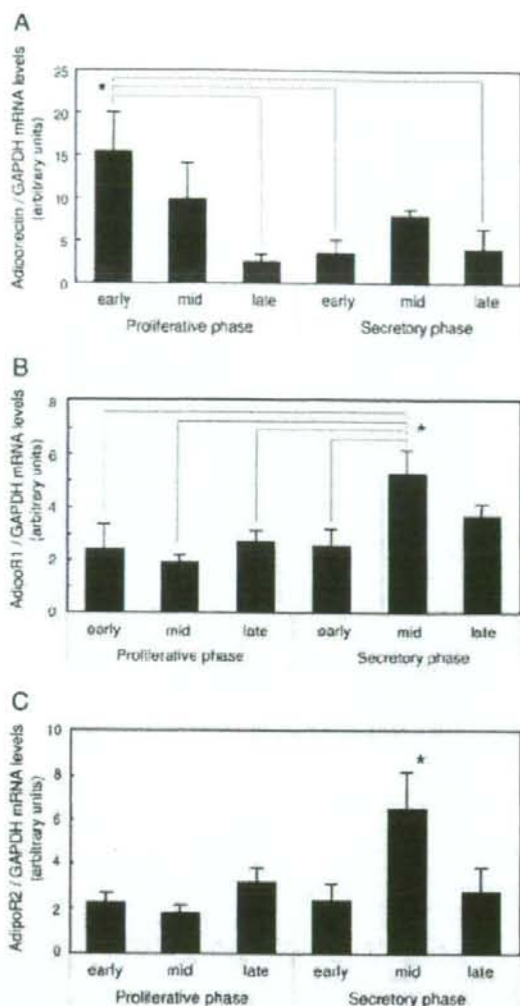

FIG. 2. Expression of adiponectin (A), AdipoR1 (B), and AdipoR2 (C) mRNA in the endometrium throughout the menstrual cycle. Endometrial tissues were obtained from 28 women (early proliferative and mid-secretory phase, n = 4; mid and late proliferative and early and late secretory phase, n = 5). Total RNA isolated from the endometrial tissues was reverse transcribed and amplified by real-time PCR using primers for adiponectin, AdipoR1, or AdipoR2. The data were calculated by subtracting the signal threshold cycles (C_T) of the internal standard (GAPDH) from the C_T of adiponectin, AdipoR1, or AdipoR2. Values are the mean ± SEM. A, *, $P < 0.01$ *vs.* late proliferative and early and late secretory phase; B, *, $P < 0.01$ *vs.* early, mid, and late proliferative, and early secretory phase; C, *, $P < 0.05$ *vs.* all other groups.

Expression of adiponectin, AdipoR1, and AdipoR2 mRNA in the endometrium throughout the menstrual cycle

Real-time quantitative PCR analysis showed adiponectin, AdipoR1, and AdipoR2 mRNA were expressed in endometrial tissues throughout the menstrual cycle (Fig. 2). The

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expression levels of adiponectin mRNA were significantly higher in the early proliferative phase compared with those in the late proliferative phase and the early and late secretory phases (Fig. 2A). The expression levels of AdipoR1 mRNA were significantly higher in the mid-secretory phase compared with the early, mid, and late proliferative phases and the early secretory phase (Fig. 2B). The expression levels of AdipoR2 mRNA were significantly higher in the mid-secretory phase compared with all other phases (Fig. 2C).

In vivo expression of adiponectin, AdipoR1, and AdipoR2 mRNA in the endometrium

In situ hybridization demonstrated that AdipoR1 and AdipoR2 were all expressed in glandular and luminal epithelial cells and stromal cells in all phases of the menstrual cycle (Fig. 3). Expression levels of AdipoR1 and AdipoR2 mRNA in the mid-secretory phase seemed to be higher than those in the other phases. The expression levels of each molecule appeared slightly higher in glandular and luminal epithelial cells than stromal cells. Adiponectin mRNA was also detected in glandular and luminal epithelial cells and stromal cells (Fig. 4). No specific hybridization products were observed when using the sense riboprobes.

Expression of AdipoR1 and AdipoR2 proteins in endometrial tissues, EECs, and ESCs

Using Western blotting, the expression of AdipoR1 and AdipoR2 proteins were detected as a bond at 42.4 and 35.4

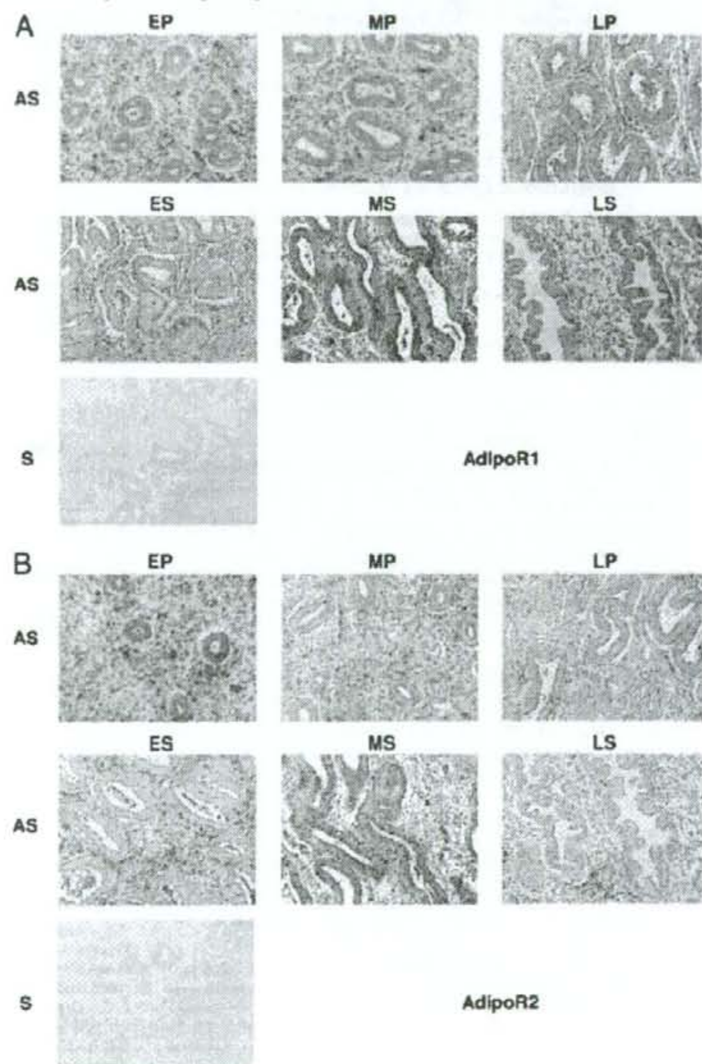

FIG. 3. *In situ* hybridization for AdipoR1 (A) and AdipoR2 (B) in the human endometrium throughout the menstrual cycle. Endometrial tissues obtained from 24 women [early (EP), mid (MP), and late proliferative (LP) phase, n = 3; early (ES) and late secretory (LS) phase, n = 4; mid-secretory (MS) phase, n = 7] were analyzed. The endometrial sections were hybridized with DIG-labeled antisense (AS) or sense (S) riboprobes. The pictures shown are representatives in each phase (AS) and MP (S). Magnification, ×100.

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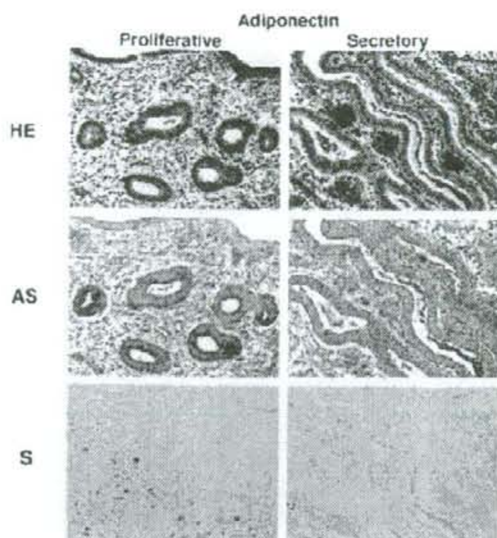

FIG. 4. *In situ* hybridization for adiponectin in the human endometrium. Endometrial sections were stained with hematoxylin and eosin (HE) or hybridized with DIG-labeled antisense (AS) or sense (S) riboprobes. The sections are of proliferative and secretory phases of the menstrual cycle. Magnification, ×100.

kDa, respectively, in endometrial tissues, EECs, and ESCs (Fig. 5).

Adiponectin-induced AMPK phosphorylation in ESCs and EECs

As shown in Fig. 6A, phosphorylation of AMPK was induced by adiponectin in ESCs in a dose-dependent manner. The phosphorylation of AMPK was apparent at 5 min and most prominent at 10 min (Fig. 6B). The phosphorylation levels were lower with 50 $\mu g/ml$ adiponectin than with 1 mM AICAR, a positive control (Fig. 6C). Phosphorylation of AMPK was also induced by 50 $\mu g/ml$ adiponectin in EECs (Fig. 6D).

Effects of adiponectin on IL-1β-induced IL-6, IL-8, and MCP-1 production in ESCs

Time-course experiments were conducted to determine the effect of adiponectin on the production of IL-6, IL-8, and MCP-1 in ESCs (Fig. 7). The concentrations of IL-6, IL-8, and

FIG. 5. Expression of AdipoR1 and AdipoR2 proteins in endometrial tissues, EECs, and ESCs. Expression of AdipoR1 and AdipoR2 proteins were examined by Western blotting in endometrial tissues, EECs, and ESCs. The result is representative of six (AdipoR1) or nine (AdipoR2) separate experiments using samples from different women. Lane T, endometrial tissues; lane E, EECs; lane S, ESCs.

MCP-1 in all samples were above the lower limits of the assays. Preincubation with adiponectin significantly decreased IL-1β-induced IL-6, IL-8, and MCP-1 production in ESCs with time, up to 24 h in culture, compared with the controls. Significant decreases were seen at 8 h for IL-8 and 12 h for IL-6 and MCP-1.

Discussion

The present study demonstrated the expression of AdipoR1 and AdipoR2 in the human endometrium for the first time. RT-PCR analysis of the endometrial tissues revealed that the gene expression of both AdipoR1 and AdipoR2 was significantly increased in the mid-secretory phase of the menstrual cycle. Histologically, the expression of both genes was equally observed in ESCs and in EECs. In cultured ESCs, adiponectin stimulated AMPK phosphorylation and suppressed IL-1β-induced IL-6, IL-8, and MCP-1 secretion. Adiponectin also induced AMPK phosphorylation in EECs.

The regulation of adiponectin receptors may be complex and varies depending on the cells and tissues. To date, sev-

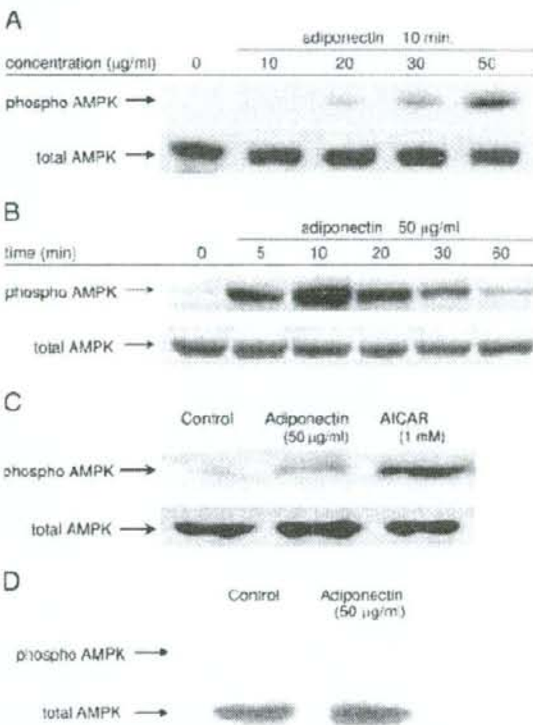

FIG. 6. Phosphorylation of AMPK by adiponectin in ESCs and EECs. Adiponectin-induced phosphorylation of AMPK was examined by Western blot analysis. Cell lysates of ESCs (A–C) and EECs (D) treated with the indicated doses of adiponectin for 10 min or for the indicated times with 50 $\mu g/ml$ adiponectin underwent Western blotting using the specific antibodies for phospho-AMPK and total AMPK. AICAR was used as a positive control (C). The results are representative of at least four separate experiments using samples from different women.

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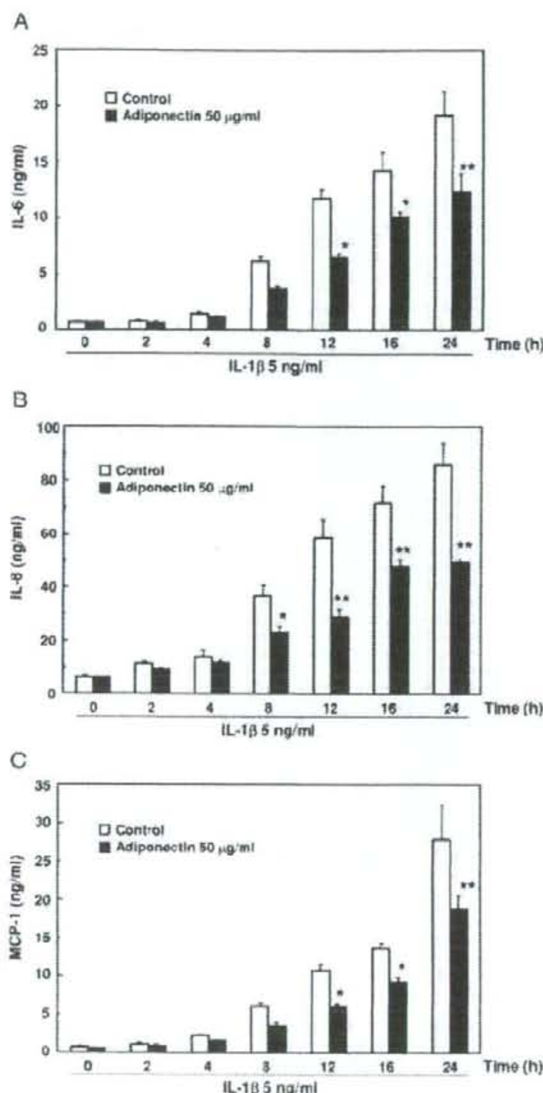

FIG. 7. Effects of adiponectin on the production of IL-6 (A), IL-8 (B), and MCP-1 (C) induced by IL-1β in ESCs. ESCs were incubated with or without adiponectin (50 μg/ml) for 24 h and then stimulated with IL-1β (5 ng/ml) for the indicated time. At the end of the incubation period, the conditioned media were collected and assayed for concentrations of IL-6, IL-8, and MCP-1 by ELISA. Values are the mean ± SEM of quadruplicate cultures. *, $P < 0.005$ vs. control; **, $P < 0.0001$ vs. control. The results are representative of at least three separate experiments using samples from different women.

eral hormones and drugs, such as insulin, GH, fenofibric acid, and troglitazone, have been shown to up- or down-regulate the expression of AdipoR1 and AdipoR2 (12, 26–29). In the present study, both AdipoR1 and AdipoR2 expression in the endometrium was increased in the midluteal phase. The midluteal phase is a period when the endometrium is receptive for the embryo and thus is called the implantation period. A myriad of genes are specifically expressed in the endometrium during the implantation period (30–32). Taken together, the increase of AdipoR1 and AdipoR2 may be a part of endometrial change for implantation, and it is intuitively speculated that the increase is affected by ovarian hormones and various cytokines that alter characteristics of the endometrium during the period.

AMPK is a fuel sensor to regulate cellular energy balance, and it also mediates effects of adipokines in modulating food intake, body weight, and glucose and lipid homeostasis (33). Adiponectin stimulates phosphorylation and activation of AMPK in the skeletal muscle, and the activated AMPK subsequently induces fatty-acid oxidation and glucose uptake. Adiponectin also stimulates AMPK in the liver and promotes fatty-acid oxidation and suppresses gluconeogenesis (10). In view of these findings, the present finding that adiponectin induced phosphorylation of AMPK in ESCs and EECs suggests that adiponectin may regulate energy supply in ESCs and EECs for an endometrial function such as reception of the embryo.

In the present study, a considerably high concentration (50 μg/ml) of adiponectin induced apparently small levels of phosphorylation of AMPK. There remains the possibility that the responses observed were cross-ligand activations.

Adiponectin has been indicated to have antiinflammatory properties. Although adiponectin inhibits phagocytic activity and LPS-induced production of TNF-α and IL-6 in macrophages (9, 34), it increases the production of the antiinflammatory mediators IL-10 and IL-1RA in monocyte, macrophage, and dendritic cells (34, 35). Antiinflammatory function of adiponectin is also suggested by its inhibiting LPS-induced IL-6 production in adipocytes (36). The present finding that adiponectin suppressed IL-1β-induced secretion of IL-6, IL-8, and MCP-1 in ESCs suggests antiinflammatory roles of adiponectin in the endometrium.

Whereas the process of implantation entails inflammation-like events (37–40), exaggerated inflammatory responses may perturb the integrity of endometrial function and lead to pathological conditions, including abortion and complicated pregnancies, such as preeclampsia and underdevelopment of the fetus. To render the endometrium favorable for implantation and ensuing fetal development, inflammatory responses of endometrial cells are suggested to be spatiotemporally fine tuned (22). In this context, the increased expression of AdipoR1 and AdipoR2 in the mid-secretory phase may subserve implantation, augmenting the adiponectin action of suppressing the production of inflammatory cytokines. However, adiponectin does not seem indispensable for pregnancy, because adiponectin-deficient mice were shown to be fertile (41).

The antiinflammatory effect of adiponectin in ESCs may be relevant to the pathogenesis of endometriosis. We have recently shown that serum and peritoneal fluid adiponectin levels are decreased in women with endometriosis (17, 42). Inflammation associated with endometriosis is suggested to promote the development of the disease. In particular, IL-1β-induced production of IL-6, IL-8, and MCP-1 in ESCs

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is suggested to be involved in the pathogenesis of the disease (22, 25). Therefore, our finding that adiponectin suppressed IL-1β-induced production of the proinflammatory molecules may explain, in part, why adiponectin levels are decreased in women with endometriosis. The inverse association between blood adiponectin levels and endometrial cancer risk (18, 19) might also be relevant to the antiinflammatory effect of adiponectin, given that inflammation is a possible promoting factor of carcinogenesis (43).

In addition to the expression of AdipoR1 and AdipoR2, we detected the expression of adiponectin in endometrial tissue. The expression levels are highest in the early proliferative phase. In light of the antifibrotic properties of adiponectin reported in the liver (44), it can be speculated that the increase in the early proliferative phase might be a protective response for fibrosis-free repair of the endometrium after shedding. However, because the expression levels of adiponectin in the endometrium were far below those in the adipose tissue (data not shown), a physiological implication of locally produced adiponectin in the endometrium is uncertain. Nevertheless, the increase in AdipoR1 and AdipoR2 levels in the implantation period is suggested to enhance adiponectin actions in the endometrium, considering that serum adiponectin levels do not show significant variation during the menstrual cycle (17).

In summary, we detected the increased expression of AdipoR1 and AdipoR2 in the human endometrium in the implantation period. In addition, adiponectin induced AMPK phosphorylation and suppressed IL-1β-induced secretion of IL-6, IL-8, and MCP-1 in cultured endometrial cells, suggesting that adiponectin may play physiological and pathological roles in the endometrium.

Acknowledgments

We thank Emi Nose for her technical assistance.

Received November 29, 2005. Accepted March 27, 2006.

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Y.Takem., Y.O., T.Yam., M.K., M.H., T.H., C.M., Y.H., O.Y., K.K., T.Yan., T.K., and Y.Taket. have nothing to declare.

References

1. **Wong GW, Wang J, Hug C, Tsao TS, Lodish HF** 2004 A family of Acrp30/adiponectin structural and functional paralogs. Proc Natl Acad Sci USA 101:10302–10307
2. **Hu E, Liang P, Spiegelman BM** 1996 AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271:10697–10703
3. **Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K** 1996 cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 221:286–289
4. **Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M** 1996 Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem (Tokyo) 120:803–812
5. **Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF** 1995 A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270:26746–26749
6. **Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T** 2001 The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941–946
7. **Brakenhielm E, Veitonmaki N, Cao R, Kihara S, Matsuzawa Y, Zhivotovsky B, Funahashi T, Cao Y** 2004 Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci USA 101:2476–2481
8. **Goldstein BJ, Scalia R** 2004 Adiponectin: a novel adipokine linking adipocytes and vascular function. J Clin Endocrinol Metab 89:2563–2568
9. **Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y** 2000 Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 96:1723–1732
10. **Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T** 2003 Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769
11. **Caminos JE, Nogueiras R, Gallego R, Bravo S, Tovar S, Garcia-Caballero T, Casanueva FF, Dieguez C** 2005 Expression and regulation of adiponectin and receptor in human and rat placenta. J Clin Endocrinol Metab 90:4276–4286
12. **Fasshauer M, Klein J, Kralisch S, Klier M, Lossner U, Bluher M, Paschke R** 2004 Growth hormone is a positive regulator of adiponectin receptor 2 in 3T3-L1 adipocytes. FEBS Lett 558:27–32
13. **Kharroubi I, Rasschaert J, Eizirik DL, Cnop M** 2003 Expression of adiponectin receptors in pancreatic β cells. Biochem Biophys Res Commun 312:1118–1122
14. **Luo XH, Guo LJ, Yuan LQ, Xie H, Zhou HD, Wu XP, Liao EY** 2005 Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. Exp Cell Res 309:99–109
15. **Staiger H, Kaltenbach S, Staiger K, Stefan N, Fritsche A, Guirguis A, Peterfi C, Weisser M, Machicao F, Stumvoll M, Haring HU** 2004 Expression of adiponectin receptor mRNA in human skeletal muscle cells is related to in vivo parameters of glucose and lipid metabolism. Diabetes 53:2195–2201
16. **Mitchell M, Armstrong DT, Robker RL, Norman RJ** 2005 Adipokines: implications for female fertility and obesity. Reproduction 130:583–597
17. **Takemura Y, Osuga Y, Harada M, Hirata T, Koga K, Morimoto C, Hirota Y, Yoshino O, Yano T, Taketani Y** 2005 Serum adiponectin concentrations are decreased in women with endometriosis. Hum Reprod 20:3510–3513
18. **Dal Maso L, Augustin LS, Karalis A, Talamini R, Franceschi S, Trichopoulos D, Mantzoros CS, La Vecchia C** 2004 Circulating adiponectin and endometrial cancer risk. J Clin Endocrinol Metab 89:1160–1163
19. **Petridou E, Mantzoros C, Dessypris N, Koukoulomatis P, Addy C, Voulgaris Z, Chrousos G, Trichopoulos D** 2003 Plasma adiponectin concentrations in relation to endometrial cancer: a case-control study in Greece. J Clin Endocrinol Metab 88:993–997
20. **Harada M, Osuga Y, Hirota Y, Koga K, Morimoto C, Hirata T, Yoshino O, Tsutsumi O, Yano T, Taketani Y** 2005 Mechanical stretch stimulates interleukin-8 production in endometrial stromal cells: possible implications in endometrium-related events. J Clin Endocrinol Metab 90:1144–1148
21. **Hirota Y, Osuga Y, Hirata T, Koga K, Yoshino O, Harada M, Morimoto C, Nose E, Yano T, Tsutsumi O, Taketani Y** 2005 Evidence for the presence of protease-activated receptor 2 and its possible implication in remodeling of human endometrium. J Clin Endocrinol Metab 90:1662–1669
22. **Yoshino O, Osuga Y, Hirota Y, Koga K, Hirata T, Yano T, Ayabe T, Tsutsumi O, Taketani Y** 2003 Endometrial stromal cells undergoing decidualization down-regulate their properties to produce proinflammatory cytokines in response to interleukin-1β via reduced p38 mitogen-activated protein kinase phosphorylation. J Clin Endocrinol Metab 88:2236–2241
23. **Noyes RW, Hertig AT, Rock J** 1950 Dating the endometrial biopsy. Fertil Steril 1:3–9
24. **Hirata T, Osuga Y, Hirota Y, Koga K, Yoshino O, Harada M, Morimoto C, Yano T, Nishii O, Tsutsumi O, Taketani Y** 2005 Evidence for the presence of toll-like receptor 4 system in the human endometrium. J Clin Endocrinol Metab 90:548–556
25. **Yoshino O, Osuga Y, Hirota Y, Koga K, Hirata T, Harada M, Morimoto C, Yano T, Nishii O, Tsutsumi O, Taketani Y** 2004 Possible pathophysiological roles of mitogen-activated protein kinases (MAPKs) in endometriosis. Am J Reprod Immunol 52:306–311
26. **Neumeier M, Weigert J, Schaffler A, Weiss T, Kirchner S, Laberer S, Scholmerich J, Buechler C** 2005 Regulation of adiponectin receptor 1 in human hepatocytes by agonists of nuclear receptors. Biochem Biophys Res Commun 334:924–929
27. **Nilsson L, Binart N, Bohlooly Y, Bramnert M, Egecioglu E, Kindblom J, Kelly PA, Kopchick JJ, Ormandy CJ, Ling C, Billig H** 2005 Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. Biochem Biophys Res Commun 331:1120–1126
28. **Tan GD, Debard C, Funahashi T, Humphreys SM, Matsuzawa Y, Frayn KN, Karpe F, Vidal H** 2005 Changes in adiponectin receptor expression in muscle and adipose tissue of type 2 diabetic patients during rosiglitazone therapy. Diabetologia 48:1585–1589
29. **Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, Kamon J, Kobayashi M, Suzuki R, Hara K, Kubota N, Terauchi Y, Froguel P, Nakae J, Kasuga M, Accili D, Tobe K, Ueki K, Nagai R, Kadowaki T** 2004 Insulin/

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Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. J Biol Chem 279:30817–30822
30. **Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, Osteen K, Taylor RN, Lessey BA, Giudice LC** 2002 Global gene profiling in human endometrium during the window of implantation. Endocrinology 143:2119–2138
31. **Mirkin S, Arslan M, Churikov D, Corica A, Diaz JI, Williams S, Bocca S, Oehninger S** 2005 In search of candidate genes critically expressed in the human endometrium during the window of implantation. Hum Reprod 20:2104–2117
32. **Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Mosselman S, Simon C** 2003 Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol Hum Reprod 9:253–264
33. **Kahn BB, Alquier T, Carling D, Hardie DG** 2005 AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1:15–25
34. **Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME** 2004 Adiponectin differentially regulates cytokines in porcine macrophages. Biochem Biophys Res Commun 316:924–929
35. **Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H** 2004 Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochem Biophys Res Commun 323:630–635
36. **Ajuwon KM, Spurlock ME** 2005 Adiponectin inhibits LPS-induced NF-κB activation and IL-6 production and increases PPARγ2 expression in adipocytes. Am J Physiol Regul Integr Comp Physiol 288:R1220–R1225
37. **Kauma SW** 2000 Cytokines in implantation. J Reprod Fertil Suppl 55:31–42
38. **Kelly RW, King AE, Critchley HO** 2001 Cytokine control in human endometrium. Reproduction 121:3–19
39. **Lindhard A, Bentin-Ley U, Ravn V, Islin H, Hviid T, Rex S, Bangsboll S, Sorensen S** 2002 Biochemical evaluation of endometrial function at the time of implantation. Fertil Steril 78:221–233
40. **Rice A, Chard T** 1998 Cytokines in implantation. Cytokine Growth Factor Rev 9:287–296
41. **Ma K, Cabrero A, Saha PK, Kojima H, Li L, Chang BH, Paul A, Chan L** 2002 Increased β-oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin. J Biol Chem 277:34658–34661
42. **Takemura Y, Osuga Y, Harada M, Hirata T, Koga K, Yoshino O, Hirota Y, Morimoto C, Yano T, Taketani Y** 2005 Concentration of adiponectin in peritoneal fluid is decreased in women with endometriosis. Am J Reprod Immunol 54:217–221
43. **Caruso C, Lio D, Cavallone L, Franceschi C** 2004 Aging, longevity, inflammation, and cancer. Ann NY Acad Sci 1028:1–13
44. **Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa J, Shinomura Y, Funahashi T, Matsuzawa Y** 2003 Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 125:1796–1807

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