0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(6):3560–3567

doi: 10.1210/jc.2004-1918

Regulation of Proliferation, Motility, and Contractility of Human Endometrial Stromal Cells by Platelet-Derived Growth Factor

Harunobu Matsumoto, Kaei Nasu, Masakazu Nishida, Hiroko Ito, Sun Bing, and Isao Miyakawa

Department of Obstetrics and Gynecology, Faculty of Medicine, Oita University, Hasama-machi, Oita 879-5593, Japan

To evaluate the involvement of platelet-derived growth factor (PDGF) isoforms (PDGF-AA, PDGF-AB, and PDGF-BB) on endometrial tissue remodeling during the perimenstrual period, we investigated the effects of PDGF on the proliferation, motility, invasiveness, and contractility of cultured human endometrial stromal cells (ESC) using a modified methylthiazoletetrazolium assay, a 5-bromo-2′-deoxyuridine incorporation assay, an *in vitro* wound repair assay, a chemotactic migration assay, a Transwell invasion assay, and a collagen gel contraction assay.

All three isoforms of PDGF significantly enhanced the cell proliferation, DNA synthesis, and *in vitro* wound repair of ESC. Chemotactic migration assay, Transwell invasion assay, and collagen gel contraction assay demonstrated that the PDGF isoforms significantly stimulated both the motility of ESC and the collagen gel contractility of ESC. PDGF-BB showed the strongest effects on these cellular functions of ESC.

The present study suggested that PDGF isoforms may promote endometrial tissue repair by enhancing the proliferation and expansion of ESC, stimulating ESC migration, and stimulating the contraction of the collagen gel matrix by ESC. By regulating ESC function during the perimenstrual period, PDGF may help to protect the endometrium from extensive fibrosis and scarring. **(*J Clin Endocrinol Metab* 90: 3560–3567, 2005)**

THE HUMAN UTERINE endometrium is a dynamic organ that undergoes cyclic phases of remarkable periodic growth, remodeling, and breakdown. Unlike most normal adult tissues, the functional layer of the uterine endometrium undergoes cyclic growth and tissue remodeling throughout the reproductive years. The remodeling of endometrial tissue, which depends on the menstrual cycle, is thought to be regulated by ovarian steroids as well as by various cytokines, neuropeptides, and growth factors, which are produced locally and secreted in an endocrine, paracrine, as well as autocrine manner (1, 2).

The tissue remodeling events that occur during menstruation share features in common with events of tissue injury and repair in other tissues, which may occur after injury or in association with various pathologies (2). However, there are important differences between tissues, and a range of features from different models are likely to apply to endometrial repair. There are also aspects of wound healing in other adult tissues that appear not to apply to the endometrium, such as the development of granulation tissue, which occurs during the healing of most cutaneous wounds, and the formation of blood clots as mediators for the initiation of repair. In addition, most wounds heal with scarring, and this is not generally seen in the case of cyclic endometrial repair in women.

First Published Online March 8, 2005

Abbreviations: BrdU, 5-Bromo-2′-deoxyuridine; ESC, endometrial stromal cells; FBS, fetal bovine serum; MTT, methylthiazoletetrazolium; PDGF, platelet-derived growth factor.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Platelet-derived growth factor (PDGF) is a member of a family of 30-kDa dimeric growth factors composed of four different polypeptide chains encoded by four different genes (3). The four PDGF chains assemble into disulfide-bonded dimers via homo- or heterodimerization; so far, five different dimeric isoforms have been described: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD (4). PDGF has been found to be produced by a number of cell types, including platelets, macrophages, endothelial cells, and smooth muscle cells (5–7), and has been shown to have several important roles in cellular proliferation, angiogenesis, inflammation, and tissue injury and its repair (8–10). PDGF plays an essential role in regulating cell growth and differentiation through its interaction with specific cell surface receptors in target tissues (11). There exist two types of PDGF receptors, denoted α and β, which differ in ligand-binding specificity; the α-receptor binds three different isoforms (PDGF-AA, PDGF-AB, and PDGF-BB) with high and comparable affinities, whereas the β-receptor binds only PDGF-BB with a similarly high affinity (12). The latter receptor type binds PDGF-AB with about a 10-fold lower affinity and appears not to recognize PDGF-AA (11). The ratio of PDGF-α receptor to PDGF-β receptor differs according to cell type (13). The production of PDGF has been reported in the human cyclic endometrium (14, 15); however, the roles of PDGF in endometrial tissue remodeling are largely unknown.

In the present study, we investigated the effects of PDGF isoforms on the proliferation, motility, invasiveness, and gel contractility of cultured human endometrial stromal cells (ESC) using well-established *in vitro* models. We also discuss herein the role of PDGF isoforms on endometrial tissue remodeling during the menstrual cycle.

Materials and Methods

ESC isolation procedure and cell culture conditions

Normal endometrial specimens were obtained from 14 premenopausal patients (aged 35–42 yr) who had undergone hysterectomies for intramural or subserosal leiomyomas. All of the specimens were diagnosed as being from the late secretory phase on the basis of the standard histological criteria. This study was approved by the Institutional Review Board of Oita University, and written informed consent was obtained from all patients. Normal ESC were separated from epithelial glands by digesting the tissue fragments with collagenase, as previously described (16). Briefly, the tissue was minced into 2- to 3-mm pieces and incubated with collagenase (200 U/ml) (Life Technologies, Inc.-BRL, Gaithersburg, MD) in DMEM (Life Technologies) with stirring for 30 min at 37 C. The suspension was filtered through a 150-μm wire sieve to remove mucus and undigested tissue. The filtrate was then passed through an 80-μm wire sieve, which allowed the stromal cells to pass through while the intact glands were retained. After the cells were washed three times in serum-free DMEM, they were transferred to culture flasks (Corning, Corning, NY) at a density of 10^6 cells/ml in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), streptomycin (100 U/ml), and penicillin (100 U/ml) (all from Life Technologies). The culture medium was replaced every 4 d. After three passages (15–20 d after isolation) using standard methods of trypsinization, the cells were shown to be more than 99% pure, as analyzed by immunocytochemical staining with antibodies to vimentin (V9), cytokeratin, factor VIII, and leukocyte common antigen (2B11+PD7/26) (all from Dako, Copenhagen, Denmark). The ratios of leukocyte common antigen-positive cells were less than 0.5%. The cultures were incubated at 37 C in an atmosphere of 5% CO_2 in air at 100% humidity. Cells isolated separately from each individual patient were used for the following experiments. Each experiment was performed in triplicate and repeated at least four times with the cells from at least four separate patients. The following experiments were performed without the addition of estrogen to the culture media; however, the concentration of estradiol in FBS was 22.43 pg/ml according to the manufacturer's information. The culture media also contained phenol red, which has been known to have estrogenic effects.

Modified methylthiazoletetrazolium (MTT) assay

The effects of PDGF isoforms on the cell proliferation of ESC were determined in 96-well plates by a modified MTT assay using WST-1 (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's protocols. A total of 5×10^4 cells of ESC in DMEM supplemented with 0.1% BSA (Sigma-Aldrich, St. Louis, MO) was distributed into each well of a 96-well flat-bottomed microplate (Corning) and incubated overnight. The medium was then removed, and the cells were incubated for 48 h with 200 μl of experimental medium (DMEM plus 0.1% BSA) containing various concentrations of PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml) (R&D Systems, Minneapolis, MN). Thereafter, 20 μl of WST-1 dye was added to each well, which was then incubated for another 4 h. Cell proliferation was evaluated by measuring the absorbance at 540 nm.

5-Bromo-2′-deoxyuridine (BrdU) incorporation assay

The effects of PDGF isoforms on the DNA synthesis of ESC were determined by BrdU incorporation using cell proliferation ELISA (Roche Diagnostics). A total of 1×10^4 cells of ESC in DMEM supplemented with 0.1% BSA was distributed into each well of a 96-well flat-bottomed microplate and incubated overnight. The medium was then removed, and the cells were incubated for 48 h with 100 μl of experimental medium containing various concentrations of PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). Thereafter, 10 μl of BrdU (10 mM) was added to each well and incubated for another 2 h. BrdU incorporation was then evaluated by measuring the absorbance at 450 nm according to the manufacturer's protocols.

In vitro wound repair assay

In vitro wound repair assays were performed as previously described (17). Cells were grown to confluence in six-well plates (Corning). Before being assayed, the cells were challenged overnight with serum-free medium. The monolayer was lesioned using a 2-mm cell scraper without damaging the dish surface. Immediately post lesion, areas for evaluation were chosen, and parallel samples were incubated in DMEM plus 0.1% BSA for 24 h with PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). The incubation time was determined by background experiments. The cells were then fixed with 3% paraformaldehyde and stained with Giemsa solution. Areas with lesions were photographed, and the wound repair was assessed by calculating the area in square micrometers between the lesion edges with the public domain Image program 1.61 developed at the National Institutes of Health (Bethesda, MD).

Chemotactic migration assay

The chemotactic activity of ESC was determined by chemotactic migration assay, as previously described (18). Specifically, 2×10^5 ESC were cultured in DMEM supplemented with 0.1% BSA on Transwell inserts with an 8-μm pore-size polycarbonate membrane (6.5 mm; Corning) with PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml) in the lower chamber. The incubation time was determined by background experiments. After 4 h, the number of cells appearing by Giemsa staining on the undersurface of the polycarbonate membranes was scored visually in six random, nonoverlapping fields at ×200 magnification using a light microscope.

Transwell invasion assay

The invasive properties of ESC were determined by Transwell invasion assay, as previously described (19, 20). In brief, polycarbonate membranes with 8-μm pores at the bottom of Transwells (6.5 mm; Corning) were coated with a thin layer of growth factor-reduced Matrigel (Becton Dickinson Labware, Bedford, MA), such that the cells were forced to degrade the Matrigel barrier before they migrated to the undersurface of the polycarbonate membranes. Specifically, 2×10^5 ESC were cultured in DMEM supplemented with 10% FBS on Matrigel-coated Transwell inserts with PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). After 48 h, the number of cells appearing by Giemsa staining on the undersurface of the polycarbonate membranes was scored visually in six random, nonoverlapping fields at ×200 magnification using a light microscope. The incubation time was determined by background experiments.

Collagen gel contraction assay

Cellular collagen gel contraction assays were performed as previously described (21). A sterile solution of acid-soluble collagen type I purified from porcine tendons (Cellmatrix type I-A; Nitta Gelatin Inc., Osaka, Japan) was prepared according to the manufacturer's instructions. ESC were embedded in the collagen gel and cultured three-dimensionally. Briefly, ESC were suspended in the collagen solution (3.0×10^5 cells/ml). The collagen/cell mixture (2 ml/plate) was dispensed into 35-mm culture plates (Corning) coated with 0.2% BSA, and the mixture was allowed to polymerize at 37 C for 30 min. Immediately after polymerization, 1 ml of culture medium with PDGF-AA, PDGF-AB, or PDGF-BB (final concentration, 0.1–10 ng/ml) was added to each plate. After incubation for 48 h, the collagen gels were photographed, and the area of the gel surface was measured with the image analysis program. The incubation time was determined by background experiments.

Statistical analysis

Data are presented as the means ± SD and were analyzed by the Bonferroni/Dunn test with StatView 4.5 (Abacus Concepts, Berkeley, CA). $P < 0.05$ was accepted as statistically significant.

Results

The effects of PDGF isoforms on the cell proliferation of ESC were assessed by modified MTT assay. As shown in Fig. 1, the cell number of viable ESC was significantly increased by addition of increasing amounts of PDGF isoforms. Al-

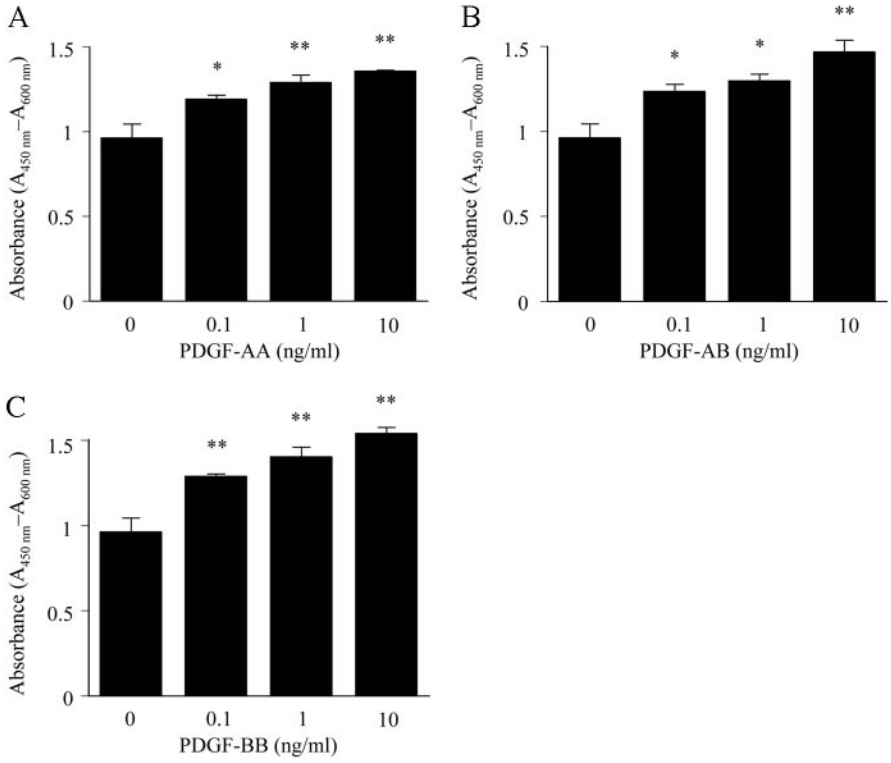

FIG. 1. The effect of PDGF isoforms on the cell proliferation of ESC as assessed by WST-1 assay. A total of 5×10^4 cells of ESC were distributed into each well of a 96-well flat-bottomed microplate and incubated overnight. The medium was then removed, and the cells were incubated for 48 h with 200 μl of experimental medium containing various concentrations of PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). Thereafter, 20 μl of WST-1 dye was added to each well and incubated for another 4 h. Cell proliferation was evaluated by measuring the absorbance at 540 nm. Representative results are shown. *, $P < 0.001$; **, $P < 0.0001$ *vs.* untreated controls (Bonferroni/Dunn test).

though it was not statistically significant, PDGF-BB tended to show the strongest effect among the three isoforms [$P < 0.0005$ *vs.* PDGF-AA (10 ng/ml)-stimulated group, but not significant *vs.* PDGF-AB (10 ng/ml)-stimulated group].

To further assess the effects of PDGF isoforms on the cell proliferation, DNA synthesis of ESC after PDGF treatment was evaluated by BrdU incorporation assay. As shown in Fig. 2, DNA synthesis of ESC was significantly increased by the addition of increasing amounts of PDGF isoforms. PDGF-BB showed the strongest effect among the three isoforms [$P <$

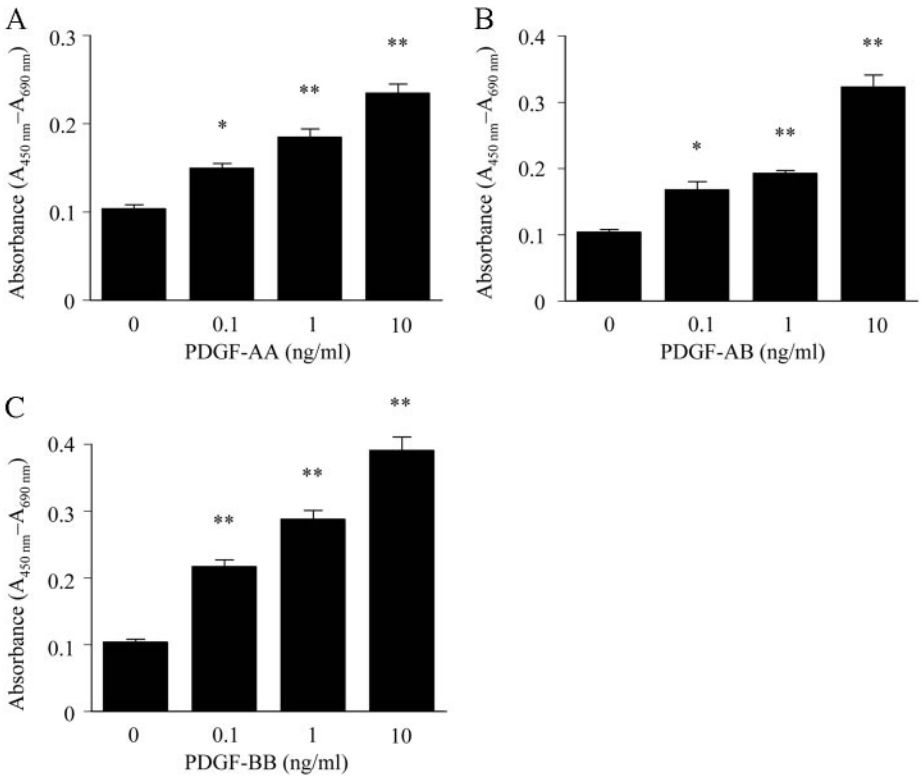

FIG. 2. The effect of PDGF isoforms on the DNA synthesis of ESC as assessed by BrdU incorporation assay. A total of 1×10^4 cells of ESC in DMEM supplemented with 0.1% BSA was distributed into each well of a 96-well flat-bottomed microplate and incubated overnight. The medium was then removed, and the cells were incubated for 48 h with 100 μl of experimental medium containing various concentrations of PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). Thereafter, 10 μl of BrdU (10 mM) was added to each well and incubated for another 2 h. BrdU incorporation was then evaluated by measuring the absorbance at 450 nm. Representative results are shown. *, $P < 0.0005$; **, $P < 0.0001$ *vs.* untreated controls (Bonferroni/Dunn test).

0.0001 *vs.* PDGF-AA (10 ng/ml)-stimulated group and PDGF-AB (10 ng/ml)-stimulated group].

The effects of PDGF isoforms on the motility of ESC were assessed by *in vitro* wound repair assay. After 24 h, 19.7 ± 0.3% of the wounded area was repaired by ESC under untreated conditions. As shown in Fig. 3, wound repair of ESC was significantly enhanced by the addition of increasing amounts of PDGF isoforms. PDGF-BB showed the strongest effect among the three isoforms [$P < 0.0001$ *vs.* PDGF-AA (10 ng/ml)-stimulated group and PDGF-AB (10 ng/ml)-stimulated group].

Next, the effects of PDGF isoforms on the chemotactic activity of ESC were assessed by chemotactic migration assay. After 4 h, 54.0 ± 2.0 ESC per six fields had migrated after 4 h under untreated conditions. As shown in Fig. 4, PDGF isoforms significantly stimulated the chemotactic migration of ESC. PDGF-BB showed the strongest effect among the three isoforms [$P < 0.0001$ *vs.* PDGF-AA (10 ng/ml)-stimulated group and PDGF-AB (10 ng/ml)-stimulated group].

The effects of the three PDGF isoforms on the invasive properties of ESC were investigated by *in vitro* invasion assay. After 48 h, 88.7 ± 4.1 ESC per six fields had invaded the Matrigel barrier under untreated conditions. As shown in Fig. 5, each PDGF isoform significantly enhanced the invasion of ESC in a dose-dependent manner. PDGF-BB showed the strongest effect among the three isoforms [$P < 0.0001$ *vs.* PDGF-AA (10 ng/ml)-stimulated group and PDGF-AB (10 ng/ml)-stimulated group].

Finally, the effects of the three PDGF isoforms on the collagen gel contraction of ESC were evaluated. In the pres-

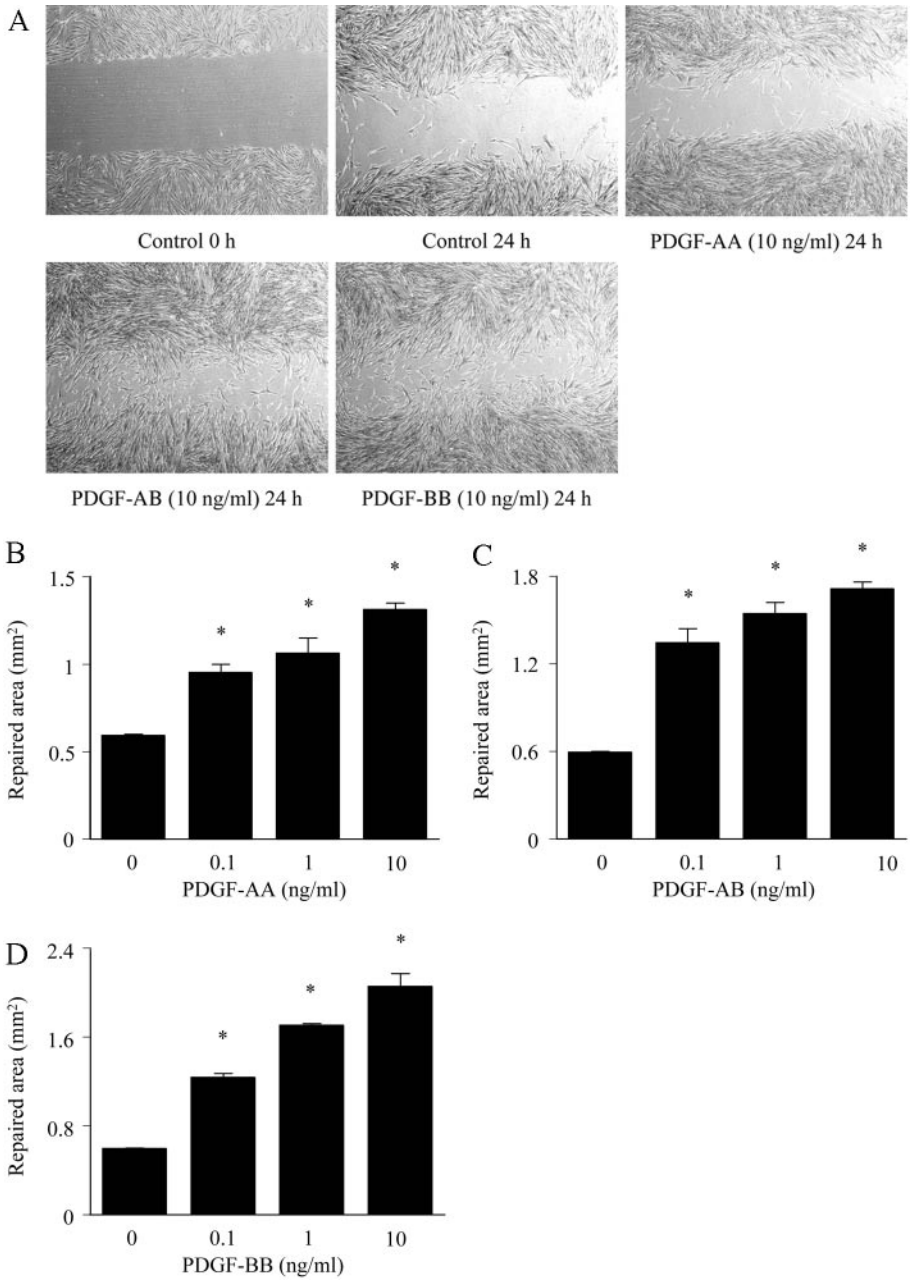

FIG. 3. The effects of PDGF isoforms on the *in vitro* wound repair of ESC. ESC grown to confluence in six-well plates were challenged overnight with serum-free medium. The monolayer was lesioned using a 2-mm cell scraper without damaging the dish surface. Immediately post lesion, areas for evaluation were chosen, and parallel samples were incubated in DMEM plus 0.1% BSA for 24 h with PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). The cells were then fixed with 3% paraformaldehyde and stained with Giemsa solution. Areas with lesions were photographed (A), and the wound repair was assessed by calculating the area in square micrometers between the lesion edges with the image analysis program (B). Representative results are shown. *, $P < 0.0001$ *vs.* untreated controls (Bonferroni/Dunn test).

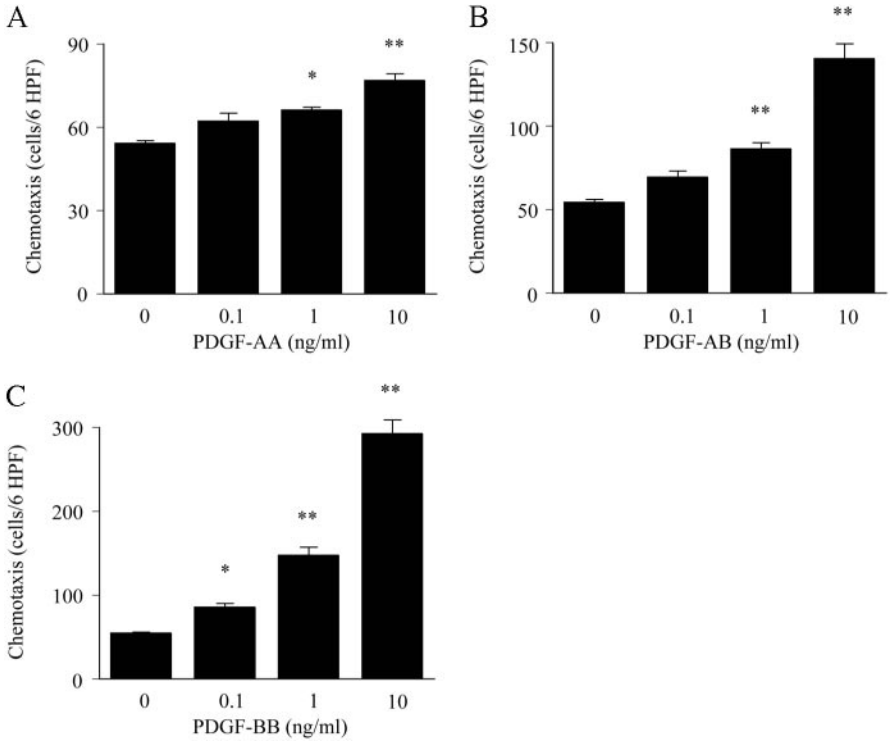

FIG. 4. The effects of PDGF isoforms on the chemotaxis of ESC as assessed by chemotactic migration assay. A total of 2×10^5 ESC were cultured in DMEM supplemented with 0.1% BSA on Transwell inserts with an 8-μm pore-size polycarbonate membrane (6.5 mm) with PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml) in the lower chamber. After 4 h, the number of cells appearing by Giemsa staining on the undersurface of the polycarbonate membranes was scored visually in six random, nonoverlapping fields at ×200 magnification using a light microscope. Representative results are shown. *, $P < 0.01$; **, $P < 0.0001$ *vs.* untreated controls (Bonferroni/Dunn test).

ence of FBS, untreated ESC showed significant collagen gel contractility (59.4 ± 0.9% contraction after 48 h *vs.* 0-h controls). As shown in Fig. 6, the areas of the gel surface after 48 h were significantly reduced in the presence of the PDGF isoforms in a dose-dependent manner (24.6, 37.5, and 38.4% reduction of the gel surface area *vs.* untreated controls at a concentration of 10 ng/ml PDGF-AA, PDGF-AB, and PDGF-BB, respectively). Although it was not statistically significant, it appeared that the PDGF-BB showed the strongest effect on the collagen gel contractility of ESC among the three isoforms [$P < 0.0001$ *vs.* PDGF-AA (10 ng/ml)-stimulated group, but not significant *vs.* PDGF-AB (10 ng/ml)-stimulated group].

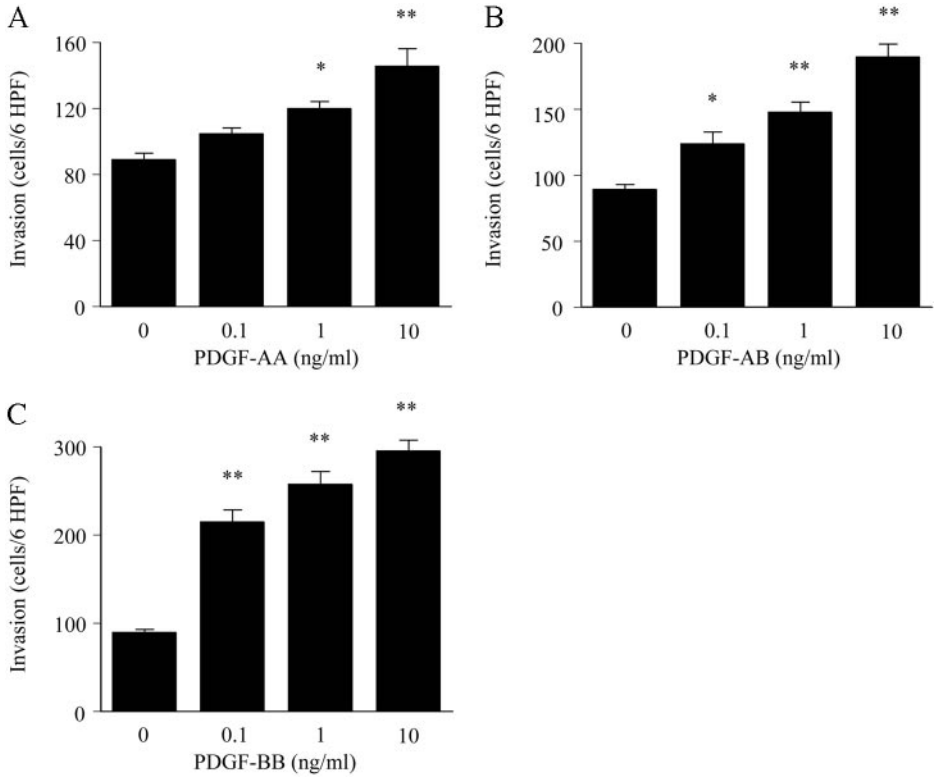

FIG. 5. The effects of PDGF isoforms on the invasion of ESC as assessed by Transwell invasion assay. A total of 2×10^5 ESC were cultured in DMEM supplemented with 10% FBS on Matrigel-coated 8-μm pore-size Transwell inserts (6.5 mm) with PDGF-AA, PDGF-AB, or PDGF-BB (0.1–10 ng/ml). After 48 h, the number of cells appearing by Giemsa staining on the undersurface of the polycarbonate membranes was scored visually in six random, nonoverlapping fields at ×200 magnification using a light microscope. Representative results are shown. *, $P < 0.001$; **, $P < 0.0001$ *vs.* untreated controls (Bonferroni/Dunn test).

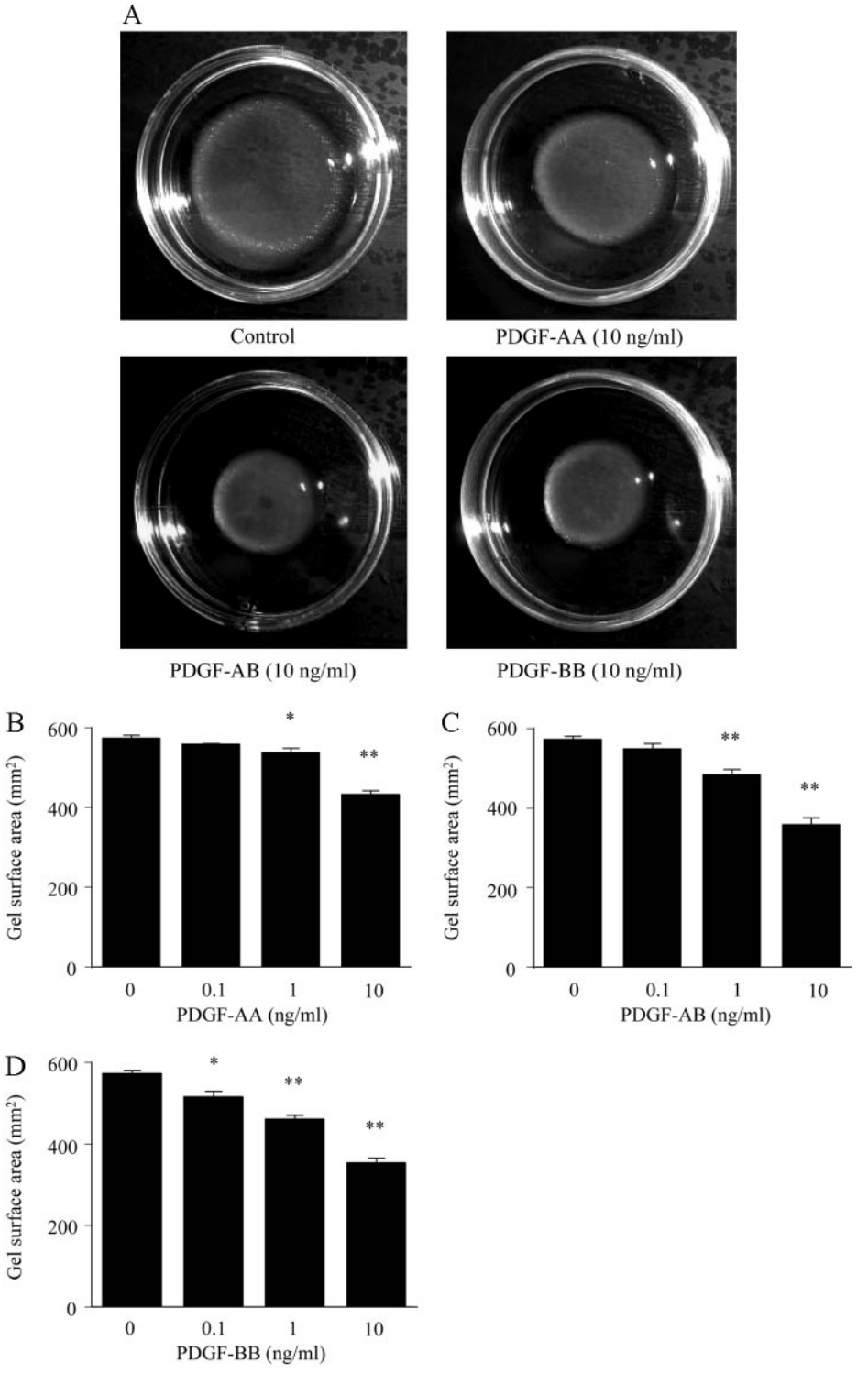

FIG. 6. The effects of PDGF isoforms on the collagen gel contractility of ESC as assessed by cellular collagen gel contraction assay. ESC were suspended in the sterile solution of acid-soluble collagen type I (3.0×10^5 cells/ml). The collagen/cell mixture (2 ml/plate) was dispensed into 35-mm culture plates coated with 0.2% BSA; the mixture was allowed to polymerize at 37 C for 30 min. Immediately after polymerization, 1 ml of culture medium with PDGF-AA, PDGF-AB, or PDGF-BB (final concentration, 0.1–10 ng/ml) was added to each plate. Accordingly, ESC were embedded in the collagen gel and cultured three-dimensionally. After incubation for 48 h, the collagen gels were photographed (A), and the collagen gel contractility was assessed by measuring the gel surface area with the image analysis program (B). Representative results are shown. *, $P < 0.0025$; **, $P < 0.0001$ *vs.* untreated controls (Bonferroni/Dunn test).

Discussion

During the secretory phase, the endometrium undergoes changes, including differentiation of its epithelium into secretory glands and the transformation of its stromal mesenchyme into predecidual cells. During the late secretory phase of the cycle, widespread degeneration is observed in the basal lamina supporting the decidualized endometrial cells and the endothelium of blood vessels (22). There is very rapid but incomplete degeneration of the functionalis layer, exposing open blood vessels and glands. Endometrial destruction is a consequence of the activity of matrix-degrading enzymes on the extracellular matrix (*i.e.* both fibrillar matrix and basal lamina), with the resultant loss of blood vessel integrity and shedding of most of the functionalis layer after

steroid hormone support is withdrawn at the end of each menstrual cycle (2). The menstrual bleeding stops at the time the tissue destruction has ceased. When substantial endometrial tissue loss occurs during menstruation, ESC ingrowth, extracellular matrix deposition, and angiogenesis, often in concert with tissue contraction, reestablish organ integrity. Regeneration begins in areas in which the mouths of the basal glands are free from overlying degenerated tissue, and there is simultaneous and progressive epithelial outgrowth from these glands and ingrowth from the intact peripheral surface membrane bordering the denuded basalis (2). The stromal tissue begins to grow only when the endometrial wound is completely reepithelialized. These events are thought to occur under the influence of increasing estrogen concentrations, which are probably locally regulated by a number of growth factors and other regulatory factors (2).

PDGF has been indicated to have several important roles in cellular proliferation, angiogenesis, inflammation, and tissue injury and its repair (8–10). In the human endometrium, it has been demonstrated that PDGF can induce cell proliferation of ESC (15, 23, 24), decidual cells (25), endometrial epithelial cells (26), and smooth muscle cells of spiral arteries (27). PDGF has been suggested to be produced by ESC (15) and macrophages (6) in the cyclic endometrium. Therefore, it is considered to be one of the most significant factors mediating endometrial cell dynamics by autocrine and paracrine mechanisms. However, the roles of PDGF in the endometrium other than its mitogenic properties are largely unknown. In the present study, we evaluated the effects of PDGF isoforms with respect to endometrial tissue remodeling during the menstrual cycle. PDGF isoforms significantly stimulated the proliferation of ESC, which is consistent with previous reports (15, 23, 24). We found that three isoforms of PDGF significantly enhanced the *in vitro* wound healing of ESC. On the other hand, PDGF significantly stimulated the chemotaxis, invasion, and collagen gel contraction of ESC. These findings are consistent with reported properties of PDGF on other cell types (28–34). Because estrogen has been demonstrated to up-regulate the PDGF production in other cell types (35), it is also suggested that estrogen might modulate the cellular function of ESC partially mediated by the enhanced production of PDGF by these cells.

The concentrations of PDGF isoforms (0.1–10 ng/ml) in the present study were chosen according to the *in vitro* studies reported previously (15, 23, 25, 26). To our knowledge, there has been no report that evaluated the endometrial tissue concentrations of PDGF. However, Morita *et al.* (27) reported that the serum PDGF levels of pregnant women were 2–3 ng/ml. Taking these findings together, the PDGF concentrations used in this study were considered to be physiological.

Fibroplasia is one of the essential components of tissue repair when substantial endometrial tissue has been shed during menstruation. Because scarless repair is one of the characteristics of endometrial remodeling, PDGF may control the motility of ESC during the peri-menstrual period to avoid excessive fibroplasia. Our present findings suggest that PDGF may contribute to tissue remodeling during perimenstruation by regulating the process of endometrial wound healing after menstrual shedding.

Another important event during endometrial wound healing is the contraction of connective tissue, which is carried out by fibroblastic cells. ESC appear to be responsible for wound contraction; however, the signals that stimulate contraction in the endometrium are largely unknown. Collagen gel contraction by fibroblasts has been ascribed to the contraction of actin filaments, which generate the cumulative traction force exerted by fibroblasts on collagen fibrils (36). *In vitro* collagen gel contraction is considered to be equivalent to the wound contraction process *in vivo* (37). In the presence of serum, the degree of collagen gel contraction was found to be influenced by cell spreading, cell movement, and reorganization of the collagen gel (37, 38). Because of the similarities between the *in vitro* system and contracting wound tissue, Bell *et al.* (37) have proposed that fibroblast-contracted collagen gel represents a tissue-equivalent construct (37). In the present study, we demonstrated that PDGF isoforms could stimulate ESC to contract the collagen gel matrix. These results are similar to those of previous reports that examined the effects of PDGF on other cell types (28, 30, 34). The contractile activity of ESC may favor expulsion of the endometrium during menstruation, reduce the size of the endometrial wound defect for eventual wound closure, and reduce the amount of fibroplasia and angiogenesis necessary for the reestablishment of organ integrity. Furthermore, PDGF-induced collagen gel contraction by ESC was considered to be distinguishable from PDGF-induced enhancement of the motility of ESC.

Taking these findings into account, it appears that PDGF isoforms promote endometrial tissue repair through at least three distinct processes. First, PDGF stimulates the cell proliferation of ESC. Second, PDGF stimulates the chemotactic migration and invasion of ESC. During the endometrial wound healing process, such mesenchymal cell ingrowth *in vivo* can help to repopulate the space created by tissue loss. However, excessive ESC ingrowth during the perimenstrual period can result in the invasion of a physiologically functional space. Third, PDGF stimulates the ESC-driven contraction of the collagen gel matrix, as demonstrated in this report. During endometrial wound repair after menstrual shedding, tissue contraction can help in reducing the amount of fibroplasia needed to reestablish organ integrity.

Interestingly, PDGF-BB showed the strongest effects on the cellular functions of ESC. Although we have not examined the expression of receptors for PDGF in ESC, it has been reported that PDGF-β receptor is predominantly expressed in ESC (15). PDGF-β receptor binds only PDGF-BB with high affinity (12). Recently, it has been shown that human uterine tissue expresses significant amounts of PDGF-BB (14). Although the effects of exogenous PDGF treatment on the endogenous PDGF expression and the corresponding receptor expression in ESC are not yet elucidated, these findings may be the underlying mechanisms of our present observations.

In summary, we demonstrated that all three relevant PDGF isoforms enhanced the proliferation and *in vitro* wound repair of ESC and stimulated chemotactic migration and invasion of these cells. In addition, these cytokines were also found to stimulate the contractility of ESC. The present

results therefore suggested that PDGF may be involved in the cyclic tissue remodeling of the endometrium and may protect the endometrium from extensive fibrosis and scarring by regulating these differential ESC functions. PDGF may thus play a number of important roles in endometrial tissue remodeling during the perimenstrual period.

Acknowledgments

Received September 28, 2004. Accepted February 25, 2005.

Address all correspondence and requests for reprints to: Kaei Nasu, M.D., Ph.D., Department of Obstetrics and Gynecology, Faculty of Medicine, Oita University, Hasama-machi, Oita, 879-5593, Japan. E-mail: nasu@med.oita-u.ac.jp.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (16591672 to K.N and 13671733 to I.M.) and by a grant from the Yamaguchi Endocrine Research Association (K.N.).

References

1. **Tanaka T, Umesaki N, Mizuno K, Chang L, Miyama M, Ohtaki S, Ogita S** 1998 Enhancement of apoptotic susceptibility in human endometrial epithelial cell line HHUA by transforming growth factor-β1. Horm Metab Res 30:61–65
2. **Salamonsen LA** 2003 Tissue injury and repair in the female human reproductive tract. Reproduction 125:301–311
3. **Fredriksson L, Li H, Eriksson U** 2004 The PDGF family: four gene products form five dimeric isoforms. Cytokine Growth Factor Rev 15:197–204
4. **Johnsson A, Heldin CH, Westermark B, Wasteson A** 1982 Platelet-derived growth factor: identification of constituent polypeptide chains. Biochem Biophys Res Commun 104:66–74
5. **Heldin CH, Wasteson A, Westermark B** 1985 Platelet-derived growth factor. Mol Cell Biol 39:169–187
6. **Shimokado K, Raines EW, Madtes DK, Barrett TB, Benditt EP, Ross R** 1985 A significant part of macrophage-derived growth factor consists of at least two forms of PDGF. Cell 43:277–286
7. **Ross R, Raines EW, Bowen-Pope DF** 1986 The biology of platelet-derived growth factor. Cell 46:155–169
8. **Ross R, Glomset JA** 1973 Arteriosclerosis and the arterial smooth muscle cell. Science 180:1332–1339
9. **Goustin AS, Betsholtz C, Pfeifer-Ohlsson S, Persson H, Rydnert J, Bywater M, Holmgren G, Heldin CH, Westermark B, Ohlsson R** 1985 Coexpression of the sis and myc proto-oncogenes in developing human placenta suggests autocrine control of trophoblast growth. Cell 41:301–312
10. **Deuel TF** 1987 Polypeptide growth factors: role in normal and abnormal cell growth. Annu Rev Cell Biol 3:443–492
11. **Westermark B** 1990 The molecular and cellular biology of platelet-derived growth factor. Acta Endocrinol (Copenh) 123:131–142
12. **Heldin CH, Backstrom G, Ostman A, Hammacher A, Ronnstrand L, Rubin K, Nister M, Westermark B** 1988 Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. EMBO J 7:1387–1393
13. **Kazlauskas A, Bowen-Pope D, Seifert R, Hart CE, Cooper JA** 1988 Different effects of homo- and heterodimers of platelet-derived growth factor A and B chains on human and mouse fibroblasts. EMBO J 7:3727–3735
14. **Boehm KD, Daimon M, Gorodeski IG, Sheean LA, Utian WH, Ilan J** 1990 Expression of the insulin-like and platelet-derived growth factor genes in human uterine tissues. Mol Reprod Dev 27:93–101
15. **Chegini N, Rossi MJ, Masterson BJ** 1992 Platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and EGF and PDGF-B-receptors in human endometrial tissue: localization and *in vitro* action. Endocrinology 130:2373–2385
16. **Nasu K, Narahara H, Matsui N, Kawano Y, Tanaka Y, Miyakawa I** 1999 Platelet-activating factor stimulates cytokine production by human endometrial stromal cells. Mol Hum Reprod 5:548–553
17. **Bilic G, Ochsenbein-Kölble N, Hall H, Huch R, Zimmermann R** 2004 In vitro repair by human amnion epithelial and mesenchymal cells. Am J Obstet Gynecol 190:87–92
18. **Nasu K, Ishida T, Setoguchi M, Higuchi Y, Akizuki S, Yamamoto S** 1995 Expression of wild-type and mutated rabbit osteopontin in *Escherichia coli*, and their effects on adhesion and migration of P388D1 cells. Biochem J 307:257–265
19. **Nasu K, Zhou Y, McMaster MT, Fisher SJ** 2000 Upregulation of human cytotrophoblast invasion by hepatocyte growth factor. J Reprod Fertil Suppl 55:73–80
20. **Mulayim N, Savlu A, Guzeloglu-Kayisli O, Kayisli UA, Arici A** 2004 Regulation of endometrial stromal cell matrix metalloproteinase activity and invasiveness by interleukin-8. Fertil. Steril 81(Suppl 1):904–911
21. **Nishiyama T, McDonough AM, Bruns RR, Burgeson RE** 1994 Type XII and XIV collagens mediate interactions between banded collagen fibers in vitro and may modulate extracellular matrix deformability. J Biol Chem 269:28193–28199
22. **Roberts DK, Parmley TH, Walker NJ, Horbeit DV** 1992 Ultrastructure of the microvasculature in the human endometrium throughout the normal menstrual cycle. Am J Obstet Gynecol 166:1393–1406
23. **Surrey ES, Halme J** 1991 Effect of platelet-derived growth factor on endometrial stromal cell proliferation in vitro: a model for endometriosis? Fertil Steril 56:672–679
24. **Tang X, Rossi MJ, Masterson BJ, Chegini N** 1994 Insulin-like growth factor I (IGF-I), IGF-I receptors, and IGF binding proteins 1–4 in human uterine tissue: tissue localization and IGF-I action in endometrial stromal and myometrial smooth muscle cells in vitro. Biol Reprod 50:1113–1125
25. **Saji M, Taga M, Matsui H, Suyama K, Kurogi K, Minaguchi H** 1997 Gene expression and specific binding of platelet-derived growth factor and its effect on DNA synthesis in human decidual cells. Mol Cell Endocrinol 132:73–80
26. **Munson L, Upadhyaya NB, Van Meter S** 1995 Platelet-derived growth factor promotes endometrial epithelial cell proliferation. Am J Obstet Gynecol 173:1820–1825
27. **Morita H, Mizutori M, Takeuchi K, Motoyama S, Maruo T** 2001 Abundant expression of platelet-derived growth factor in spiral arteries in decidua associated with pregnancy-induced hypertension and its relevance to atherosis. Eur J Endocrinol 144:271–276
28. **Clark RA, Folkvord JM, Hart CE, Murray MJ, McPherson JM** 1989 Platelet isoforms of platelet-derived growth factor stimulate fibroblasts to contract collagen matrices. J Clin Invest 84:1036–1040
29. **Ferns GA, Sprugel KH, Seifert RA, Bowen-Pope DF, Kelly JD, Murray M, Raines EW, Ross R** 1990 Relative platelet-derived growth factor receptor subunit expression determines cell migration to different dimeric forms of PDGF. Growth Factors 3:315–324
30. **Gullberg D, Tingstrom A, Thuresson AC, Olsson L, Terracio L, Borg TK, Rubin K** 1990 β1 Integrin-mediated collagen gel contraction is stimulated by PDGF. Exp Cell Res 186:264–272
31. **Siegbahn A, Hammacher A, Westermark B, Heldin CH** 1990 Differential effects of the various isoforms of platelet-derived growth factor on chemotaxis of fibroblasts, monocytes, and granulocytes. J Clin Invest 85:916–920
32. **Westermark B, Siegbahn A, Heldin CH, Claesson-Welsh L** 1990 B-type receptor for platelet-derived growth factor mediates a chemotactic response by means of ligand-induced activation of the receptor protein-tyrosine kinase. Proc Natl Acad Sci USA 87:128–132
33. **Singer CF, Marbaix E, Lemoine P, Courtoy PJ, Eeckhout Y** 1999 Local cytokines induce differential expression of matrix metalloproteinases but not their tissue inhibitors in human endometrial fibroblasts. Eur J Biochem 259:40–45
34. **Grundstrom G, Mosher DF, Sakai T, Rubin K** 2003 Integrin $\alpha v\beta 3$ mediates platelet-derived growth factor-BB-stimulated collagen gel contraction in cells expressing signaling deficient integrin $\alpha 2\beta 1$. Exp Cell Res 291:463–473
35. **Bronzert DA, Pantazis P, ntoniades HN, Kasid A, Davidson N, Dickson RB, Lippman ME** 1987 Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines. Proc Natl Acad Sci USA 84:5763–5767
36. **Grinnell F** 1994 Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 124:401–404
37. **Bell E, Ivarsson B, Merrill C** 1979 Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro.* Proc Natl Acad Sci USA 76:1274–1278
38. **Guidry C, Grinnell F** 1985 Studies on the mechanism of hydrated collagen gel reorganization by human skin fibroblasts. J Cell Sci 79:67–81