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The Activation of Matrix Metalloproteinase-2 Induced by Protein Kinase C Alpha in Decidualization

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ABSTRACT

This study investigated the protein kinase C (PKC) and matrix metalloproteinase-2 (MMP-2) in the development of deciduomata in pseudo-pregnant and pregnant rats. The results showed that the expression of MMP-2 was significantly increased from day 2 to day 5 in pseudo-pregnancy and from day 7 to day 9 in pregnancy. To further investigate the correlation between MMP-2 and protein kinase Cα (PKCα), the expression of MMP-2 in the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-treated organotypic culture of decidual tissue was determined. The results showed that the active form of MMP-2 was significantly increased in the TPA-treated cultures. Moreover, this response was inhibited by the PKC inhibitor H7, the PKCα specific inhibitor Gö-6976 and the translation inhibitor cycloheximide, but not by the transcription inhibitor actinomycin D or the replication inhibitor mitomycin C. In addition, TPA also reversed the MMP-2 expression after by progesterone pretreatment in the primary decidual cells. These findings indicate that PKCα may play an important role in the regulation of the MMP-2 expression during decidualization. J. Cell. Biochem. 108: 547–554, 2009.

KEY WORDS: MATRIX METALLOPROTEINASE-2; PROTEIN KINASE C; DECIDUALIZATION

Decidualization has been attributed to the destruction of extra-cellular matrix and basement membrane by proteases in the substantial remodeling of the endometrium [Osteen et al., 1994]. Cysteine proteinases, serine proteinases, and matrix metalloproteinases (MMPs) may participate in this destructive process [Osteen et al., 1994; Nuttall and Kennedy, 1999]. MMPs are zinc-requiring enzymes that can degrade components of the extra-cellular matrix and are implicated in tissue remodeling. Their role in the onset of menstruation in vivo has been proven; however, the expression and functions of MMPs and tissue inhibitors of metalloproteinases (TIMPs) in vascular structures are poorly understood. Based on immunocytochemistry, MMPs have been considered to be the most important proteases involved in the reduction of the concentration of the extra-cellular matrix at implantation sites [Whiteside et al., 2001]. Among the MMPs, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have been reported as taking part in tissue regeneration and angiogenesis [Freitas et al., 1999]. Although these two MMP members present similar amino acid sequences, they have significant differences in their regulatory factors and structures. Moreover, the increased activity of these MMP members has also been demonstrated as playing an important role in decidualization [Zhao et al., 2002].

Shao-Hsuan Kao and Jer-Yuh Liu contributed equally to this work.

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MMP-2 may be involved in the matrix remodeling during decidualization [Das et al., 1997]; it has been demonstrated to be secreted consistently by endometrial stromal cells in culture and enhanced by the withdrawal of progesterone from cultures of decidualized endometrial stromal cells [Salamonsen et al., 1993]. Recently, the amount of this member of the MMPs has been reported to be increased by the reduction of cytosolic estrogen receptor/estrogen action during decidualization [Spencer et al., 1998]. It has also been demonstrated that the MMP-2 activated by membrane-type-1 matrix metalloproteinase (MT1-MMP) is associated with the attenuation of the progesterone level [Zhang et al., 2000]. Our previous studies have found that protein kinase C (PKC) is considered to activate the deciduoma development [Liu et al., 1998]. Several reports have demonstrated that MMP-2 activation may be induced by the PKC signaling pathway process in cultures of many cell lines [Uhm et al., 1996; Husain et al., 2005; Mountain et al., 2007]. However, whether the activation of PKC correlates with MMP-2 activation in decidualization remains unclear. This study employed the organotypic culture technique and quantitative gelatin zymography to evaluate the effects of various PKC activators and inhibitors, as well as transcription and translation inhibitors, on the expression of MMP-2 in endometrial decidualized tissue.

MATERIALS AND METHODS

CHEMICALS

12-*O*-tetradecanoylphorbol 13-acetate (TPA), Gö-6976, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H7), calphostin C, collagenase type III, progesterone, and deoxyribonuclease (DNase) were purchased from Sigma Chemical Co. (St. Louis, MO). Dulbecco's minimum essential medium (DMEM), Ham's F12 medium, fetal bovine serum (FBS) and antibiotics were purchased from Invitrogen Co. (Carlsbad, CA).

ANIMAL TREATMENT

Virgin Sprague–Dawley rats (National Science Council Animal Center, Taipei, Taiwan), weighing from 180 to 300 g, were kept at a constant temperature (24°C) and illuminated for 12 h daily (lights on from 05:00 to 17:00). After 7-day maintenance for adaptation to the new environment, vaginal smears were taken at 08:00–09:00 every morning. Only those rats showing two consecutive 4-day cycles were used for all animal experiments.

Pseudo-pregnancy was induced by vagino-cervical vibration with a glass rod for 1 min on the morning of estrus [Defeo, 1963]. The day of estrus was designated as day 0 of pseudo-pregnancy. Uterine decidualization was induced by endometrial scratching, twice, on the morning of day 4 of pseudo-pregnancy [Finn and Keen, 1963]. Therefore, day 4 of pseudo-pregnancy was designated as day 0 of decidualization. On days 0, 1, 2, 3, 5, 7, and 9 of decidualization, the decidualized uterine horn was removed, weighed, and quickly frozen on dry ice for tissue extraction.

For pregnancy, the female rats were placed with the males of proven fertility on 17:00–18:00 of the proestrus day of the third consecutive cycle. The conclusion of successful pregnancy was those found with vaginal sperm on the following morning. Each experiment was duplicated. The animals were decapitated on days 5, 7, 8, 9, 11, and 13 of pregnancy. On the indicated days of pregnancy the two uterine horns with conceptus were removed, weighed, and quickly frozen on dry ice for tissue extraction.

ORGANOTYPIC CULTURE

Three pieces ($4 \times 4\ mm^2$) of decidual tissue obtained from day 3 or 5 of pseudo-pregnancy were placed into each well of a 24-well culture plate containing 1 ml of DMEM. The tissues were cultured in a 5% CO_2 incubator at 37°C for 1 h and the cultured medium was discarded after incubation. In addition to incubating a well of cultured tissue with 1 ml DMEM as a control, the remaining ones were separately treated with PKC activators (TPA), inhibitors (H7, calphostin C or Gö-6976) or their combinations. The cultured medium was collected into vials and stored at −70°C, and the tissue was weighed and stored at −70°C.

QUANTITATIVE GELATIN ZYMOGRAPHY ASSAY

Since the weights of the specimen in each well of culture plates were not equal, the cultured medium (1 ml) was calibrated to contain the secretion from 100 mg of tissue from the phosphate-buffered saline (PBS) buffer. Gelatin zymography analysis were carried out as described previously [Wu et al., 2004] by loading 10 μl of the calibrated cultured media, the cytosol and the particulate fractions onto 8% SDS–PAGE containing 0.1% gelatin and then electrophoresed at 150 V for 2.5 h. Enzymes on the gels were re-natured by washing twice in a 2.5% (v/v) Triton X-100 solution with shaking for 30 min. The gels were then incubated in 200 ml reaction buffer (40 mM Tris–HCl, pH 8.0; 10 mM $CaCl_2$; 0.01% NaN_3) at 37°C for 16 h and then stained with 0.25% Coomassie brilliant blue R-250 for 30 min. The extract of a randomly chosen human breast cancer biopsy was used as a marker. After de-staining, quantitative analysis was performed by using an Alpha Imager 2000 densitometer (Alpha Innotech, San Leandro, CA). The total MMP-2 activity are calculated by active MMP-2 (62 kDa) plus proMMP-2 (72 kDa). The percent of MMP-2 activation was calculated by active form divided by total MMP-2. The results of MMPs expression were analyzed using analysis of variance (ANOVA), and the Student's *t*-test was used in group comparison, and $P < 0.05$ was considered statistically significant.

TISSUE EXTRACTION

All procedures were performed at 4°C. On days 3, 5, 7, and 9 of decidualization in pseudo-pregnant rats, the decidualized uterine tissues were slit open and the deciduomata were removed from the myometrium by gentle scraping with a glass slide. The deciduomal and myometrial tissues were homogenized separately. On days 0, 1, and 2 of decidualization, the deciduomal tissue could not be removed individually, and whole uterine tissues were homogenized. Besides, on days 9, 10, 11, and 13 of pregnancy, the conceptuses were collected and slit open. The trophoblast and fetus were removed. The decidual tissues were separated from the myometrium by gentle scraping with a glass slide. Both decidual and myometrial tissues were then homogenized separately. On days 5, 7, and 8 of pregnancy, the decidual tissue could not be removed individually, and whole uterine tissues were homogenized as above. All tissues, including decidual, myometrial, and cultured tissues, were homo-

genized in the PBS (0.14 M NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 14 mM K_2HPO_4) at a concentration of 100 mg tissue/ml PBS. The homogenates were then placed on ice for 10 min and then centrifuged at 10,000*g* for 30 min. The supernatant was collected and designated as the cytosol fraction. The precipitant was homogenized in a sodium dodecyl sulfate (SDS) buffer (20 mM Tris–HCl, pH 6.8; 1% SDS; 10% glycerol; and 0.01% bromophenol blue) at a concentration of 100 mg tissue/ml SDS buffer. The homogenates were centrifuged at 10,000*g* for 30 min. The supernatant was collected and designated as the particulate fraction. All the fractions were stored at −70°C.

PRIMARY CELL CULTURE

Cells were prepared from endometrial tissue as described previously [Arslan et al., 1995]. The endometrial tissue was digested with collagenase type III at a concentration of 45 U/ml, in the presence of 3.5 μg/ml DNase in calcium- and magnesium-free PBS, for 40 min at 37°C. The resultant digest was then filtered sequentially through 45 and 10 mm nylon filters to remove glands, and erythrocytes were removed by centrifugation on Ficoll-Paque. The resulting cells were re-suspended in a 1:1 mixture of DMEM and Ham's F12 medium with 10% charcoal-stripped FBS and antibiotics (penicillin, streptomycin, and fungizone) and transferred into 24-well plates (2×10^5 cells for each well) with or without the progesterone (1 μM). The medium was changed every 48 h. After 2 weeks, cells were photographed and treated with TPA after an additional 2 days. The TPA-treated medium was collected and stored at −20°C for subsequent analysis. This experiment was performed with three individual cell cultures.

RESULTS

INCREASE OF MMP-2 DURING THE DEVELOPMENT OF DECIDUOMATA IN VIVO

In pseudo-pregnancy, the deciduomata was unable to separate from the uterus before day 2. For this reason, whole uterine tissue extract was used for MMP-2 activity assay during day 0 to day 2. The activation of MMP-2 in developing deciduomata, whole uterine tissues (Ut) (days 0, 1, and 2), deciduomata (De), and myometrium (My) (days 3, 5, 7, and 9), was investigated by gelatin zymography assay. According to the previous study [Wu et al., 2004], three bands of gelatin hydrolytic activity at 92, 72, and 62 kDa represented proMMP-9, proMMP-2, and active form of MMP-2, respectively. During deciduomata development, as shown in Figure 1A,B, activation of MMP-2 significantly increased from day 0 to day 2 and then decreased. Moreover, the expression of activated MMP-2 during day 3 to day 9 was higher than on day 0. The total activity of MMP-2 also increased after trauma stimulation and reached maximum on day 2 (Fig. 1C).

In pregnancy, the activation of MMP-2 in developing deciduomata significantly increased from day 7 to day 9, and then decreased (Fig. 2A,B). The total activity of MMP-2 also rapidly rose, and reached maximum on day 8.

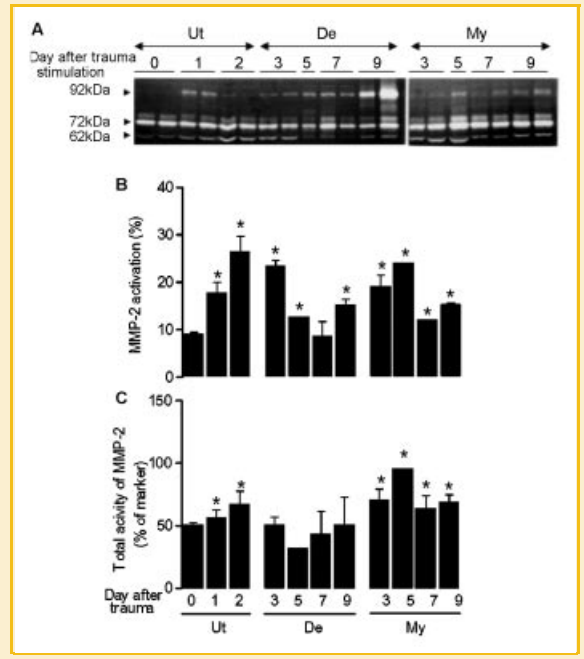

Fig. 1. The expression of MMPs in decidual uterine tissue during pseudo-pregnancy. A: The samples on days 0, 1, 2, 3, 5, 7, and 9 in the uterine tissues (Ut), deciduomata (De), or myometrium (My) were processed for zymography. The indications of 92, 72, and 62 kDa were marked. B: The quantitative data of the activation of MMP-2 were represented. C: The quantitative data of the total activity of MMP-2 were represented. Data are expressed as mean ± SEM for 3–5 measurements. $^*P < 0.05$ versus the control of day 0.

THE EFFECT OF TPA OR/AND PKC INHIBITORS ON THE EXPRESSION OF MMP-2 IN ORGANOTYPIC CULTURE OF DECIDUOMATA

The above findings indicate that the activation of MMP-2 may be associated with the development of deciduomata and parallels the down-regulation and activation of protein kinase Cα (PKCα) which has been found in previous studies [Liu et al., 1998; Shyu et al., 1999]. For investigating the correlation between PKCs and MMP-2 in deciduomata development, the experiment compared the activation and total activity of MMP-2 under PKC activator (TPA) or inhibitor (H7 and calphostin C) treatments [Parodi et al., 1990; Rotenberg et al., 1995]. As shown in Figure 3, both on day 3 and day 5, in decidual tissues, MMP-2 activation was dose-dependently increasing in TPA treatments. Under co-treatment with TPA and H7, the activity of MMP-2 was reserved in decidual tissues (day 3 and day 5), but this reservation did not present in the decidual tissues co-treated with TPA and calphostin C. In contrast, an increase in total activity of MMP-2 was shown in treatment only with calphostin C (day 5).

It has been demonstrated that the isoenzyme PKCα is activated in decidual tissues from both pseudo-pregnant and pregnant rats [Liu et al., 1998; Shyu et al., 1999]. Here, whether PKCα affected MMP-2 activation in decidual tissues was tested. The results showed that only TPA-induced secretary MMP-2 activation was inhibited by a specific PKCα inhibitor, Gö-6976; however, the inhibition was diminused when Gö-6976 concentration was in 1 nM and lower then 1 nM (Fig. 4A,B). Moreover, Gö-6976 dose-dependently inhibited

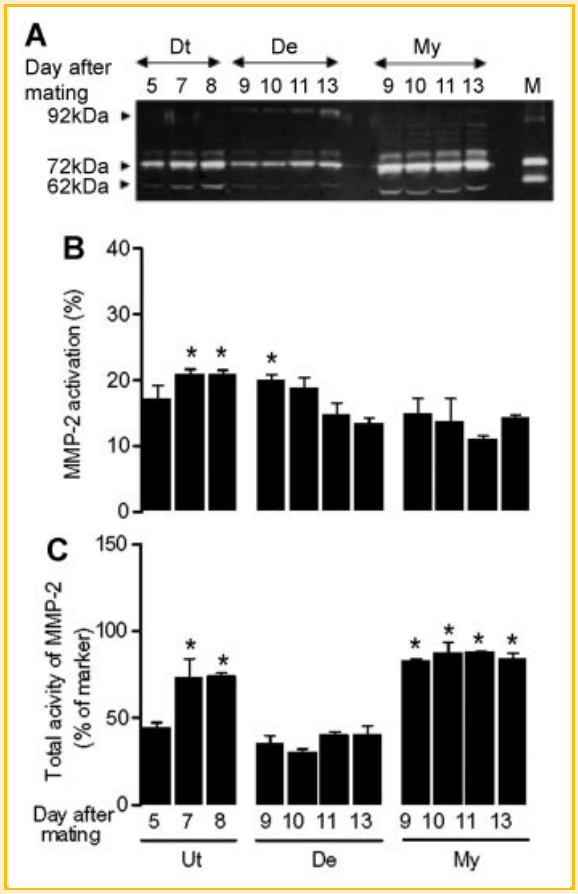

Fig. 2. The expression of MMPs in decidual uterine tissue during pregnancy. A: The samples on days 5, 7, 8, 9, 10, 11, and 13 in the uterine tissues (Ut), deciduomata (De), or myometrium (My) were processed for zymography. The indications of 92, 72, and 62 kDa were marked according to previous study. M is the extract of breast cancer tissue and defined as a marker. B: The quantitative data of the activation of MMP-2 were represented. C: The quantitative data of the total activity of MMP-2 were represented. Data are expressed as mean ± SEM for 3–5 measurements. $^{*}P < 0.05$ versus the control of day 5.

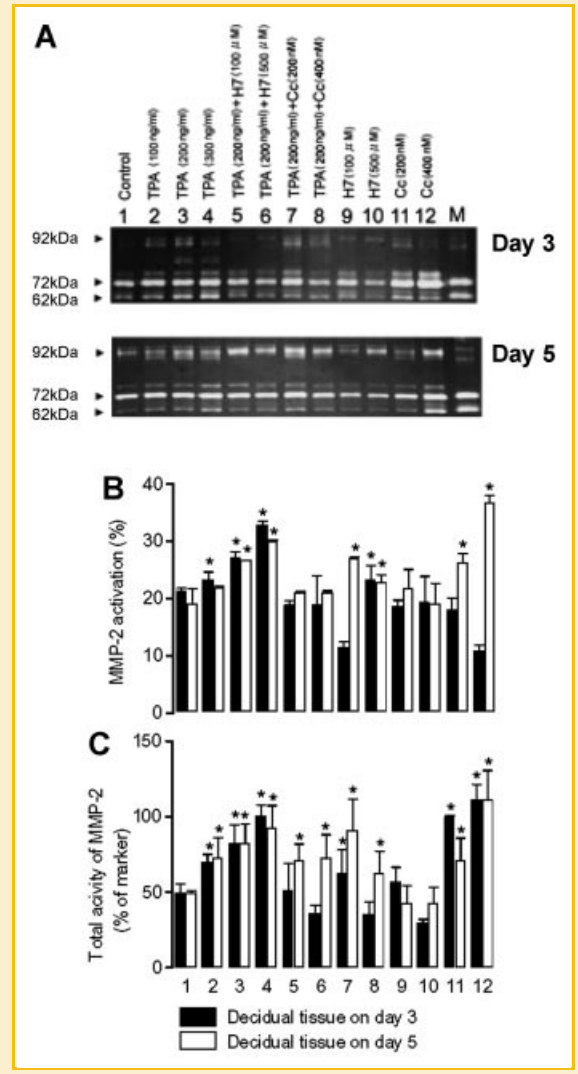

Fig. 3. The effect of TPA and/or PKC inhibitor (H7 and calphostin C) on the secretion of MMP-2 in organotypic cultures of deciduomal tissue obtained from the pseudo-pregnant rats on day 3 and day 5. A: The samples of the cultured medium were processed for zymography. The indications of 92, 72, and 62 kDa were marked according to previous study. M is the extract of breast cancer tissue and defined as a marker. B: The quantitative data of the activation of MMP-2 were represented. C: The quantitative data of the total activity of MMP-2 were represented. Data are expressed as mean ± SEM for 3–5 measurements. $^{*}P < 0.05$ versus the control of lane 1.

TPA-induced total activity of MMP-2 in secretary, cytosolic and particulate fraction (Fig. 4C). Accordingly, it is suggested that activation of PKCα might play an important role in MMP-2 activation.

THE EFFECT OF TPA OR/AND TRANSLATION AND TRANSCRIPTION FACTORS ON THE EXPRESSION OF MMP-2 IN ORGANOTYPIC CULTURE OF DECIDUOMATA

This study demonstrated that activation of PKCα was involved in the activation of MMP-2 and the increase of MMP-2 total activity in decidual tissues. The increase of MMP-2 total activity suggested that the enzyme may be different with regard to a requirement for protein synthesis. To evaluate this possibility, this experiment tested the effects of cycloheximide (CHX), actinomycin D (Act D), and mitomycin C (MMC), which inhibit protein synthesis, RNA transcription and DNA replication, respectively [Kirk, 1960; Kersten and Rauen, 1961; Obrig et al., 1971]. As shown in Figure 5, CHX almost completely inhibited TPA-induced MMP-2 activity in secretary, cytosolic and particulate fraction. In contrast, no significant variation of TPA-induced MMP-2 activity was monitored by Act D and MMC (Fig. 5).

THE EFFECT OF TPA OR/AND PROGESTERONE ON THE EXPRESSION OF MMP-2 IN PRIMARY CULTURE OF DECIDUAL CELL

Progesterone is essential for the development of endometrial receptivity for pregnancy maintenance. Many studies demonstrated that progesterone restrains endometrial tissue breakdown by inhibiting the stimulation of MMPs, which implies that the hormone

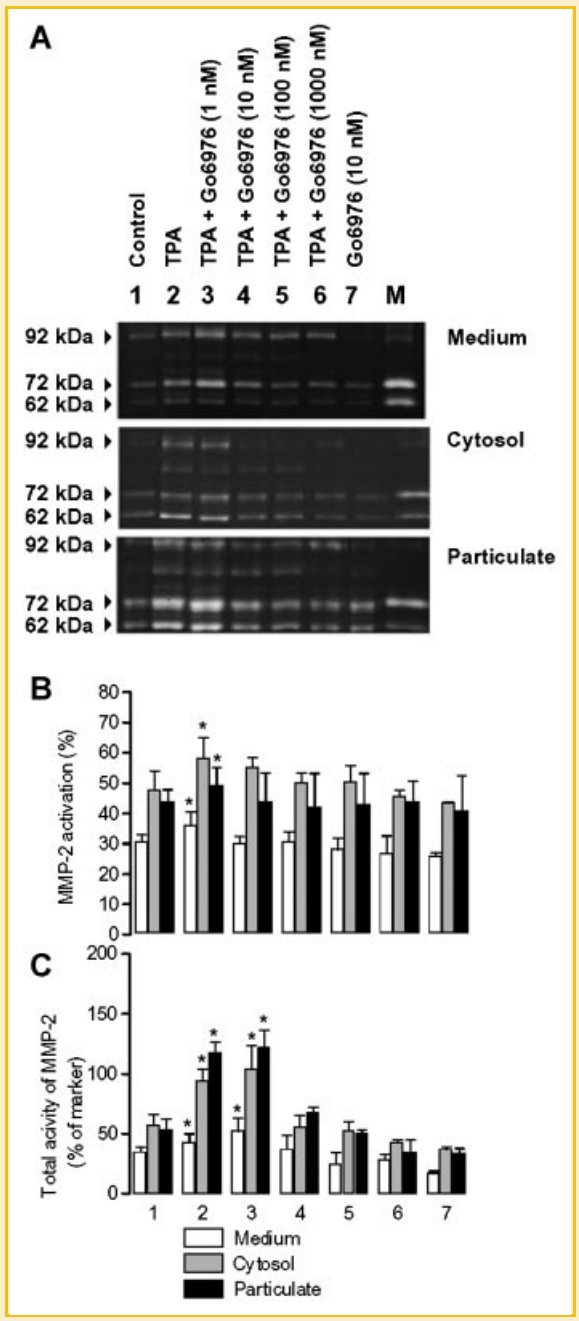

Fig. 4. The effect of TPA and/or PKC inhibitor (Gö-6976) on the secretion of MMP-2 in organotypic cultures of deciduomal tissue obtained from the pseudo-pregnant rats on day 3. A: The samples of the cultured medium were processed for zymography. The indications of 92, 72, and 62 kDa were marked according to previous study. M is the extract of breast cancer tissue and defined as a marker. B: The quantitative data of the activation of MMP-2 were represented. C: The quantitative data of the total activity of MMP-2 were represented. Data are expressed as mean ± SEM for 3–5 measurements. $^{*}P < 0.05$ versus the control of lane 1.

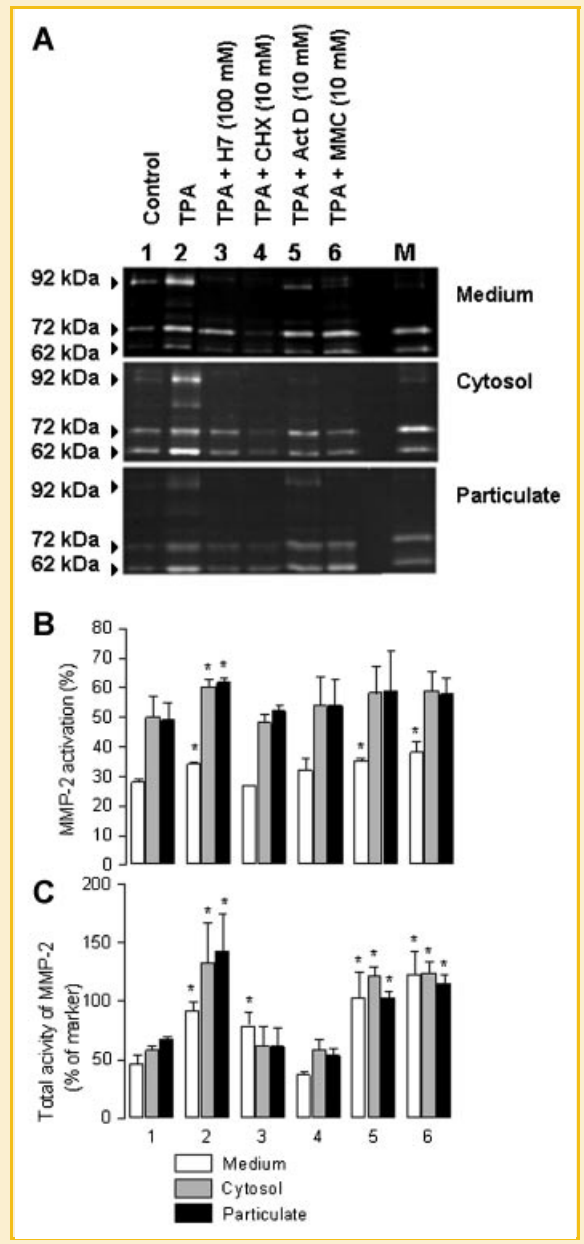

Fig. 5. The effect of TPA and/or translation inhibitor (cycloheximide—CHX), transcription inhibitor (actinomycin D—Act D), and replication inhibitor (mitomycin C—MMC) on the secretion of matrix metalloproteinases (MMP) in organotypic cultures of deciduomal tissue on day 3. A: The samples of the cultured medium were processed for zymography. The indications of 92, 72, and 62 kDa were marked according to previous study. M is the extract of breast cancer tissue and defined as a marker. B: The quantitative data of the activation of MMP-2 were represented. C: The quantitative data of the total activity of MMP-2 were represented. Data are expressed as mean ± SEM for 3–5 measurements. $^{*}P < 0.05$ versus the control of lane 1.

impedes the invasion of trophoblast cells into endometrial tissue [Goffin et al., 2003; Hashizume et al., 2003; Goldman and Shalev, 2006]. This study found that rat endometrial cells treated with 1 μM progesterone resulted in longer and thinner phenotype of decidual cells after 2 weeks (Fig. 6). By using zymography assay, the activation of MMP-2 in rat decidual cells was inhibited by progesterone, and the total activity of MMP-2 was reduced. In contrast, co-treatment with progesterone and TPA

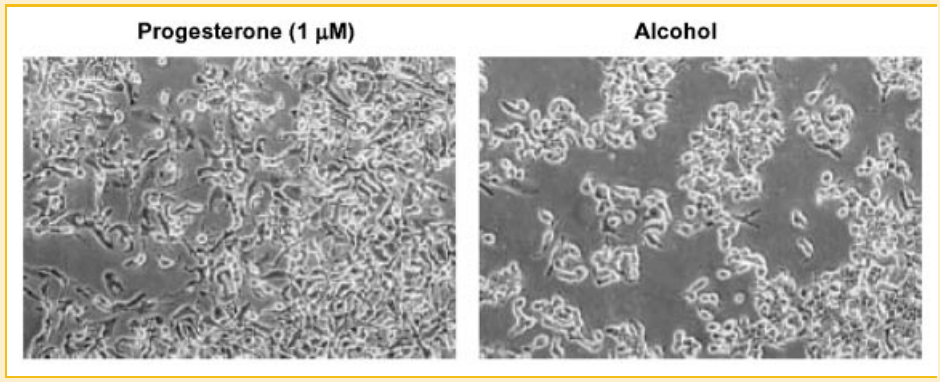

Fig. 6. Morphological change in the progesterone-treated rat decidual cells.

triggered activation of MMP-2 and increased total MMP-2 activity (Fig. 7).

DISCUSSION

The expression of activated MMP-2 in the uterine decidual tissue was significantly increased from day 2 to day 5 in pseudo-pregnancy (Fig. 1) and from day 7 to day 9 in pregnancy (Fig. 3). The difference in the times of the MMP-2 expression between pseudo-pregnant and pregnant is due to the different period of decidualization. It is suggested that this enhanced expression of activated MMP-2 in pregnancy and pseudo-pregnancy was probably the result of the spread of decidual cells [Hurst and Palmay, 1999]. The decidual cells provide protection to the embryo after implantation and supply it with nutrients in the sequential event [Golander et al., 1981]. Thus, the results confirm that the expression of MMP-2 in this period may be associated with maintaining deciduomata development.

In previous studies, the down-regulation and activation of PKCα has been found to be associated with the development of deciduomata [Liu et al., 1998; Shyu et al., 1999]. In this study, we found that the activation of MMP-2 is paralleled with the activation of PKCα in decidualization. Moreover, PKC activator TPA enhances the expression and activation of MMP-2 in organotypic culture. PKC inhibitors, especially the PKCα specific inhibitor Gö-6976, inhibit this enhanced expression. Thus, it is suggested that the activation of PKC may be involved in the regulation of the expression of MMP-2 in decidualization.

Besides, the activation of MMP-2 by TPA was not inhibited in the decidual tissues co-treated with calphostin C. In addition, an increase in total activity of MMP-2 was observed in treatment only with calphostin C. This phenomenon dose not support the previous report that the MMP-2 activity could be inhibited by the treatment of human gliomas cells with calphostin C [da Rocha et al., 2000]. However, Gamou and Shimizu [1994] reported that calphostin C does not inhibit PKC and induces the epidermal growth factor (EGF) receptor phosphorylation in the EGF receptor-hyperproducing squamous carcinoma cell line NA. These results suggest that calphostin C may appear a novel signal transduction pathway which involves MMP-2 activation, but further studies are needed.

The study indicates that the TPA-enhanced expression of MMP-2 is inhibited by the translation inhibitor CHX but not by the transcription inhibitor Act D or the replication inhibitor MMC (Fig. 5). Similarly, Matsumoto et al. [2009] reported that the MMP expression during the decidualization is not regulated by their mRNA expression after the treatment with the decidual stimuli, but by the activation of MMPs. Sharma et al. [2007] reported that in a PKC-induced, or activated, internal ribosome entry site (IRES)-*trans* acting factors regulate GATA-4 IRES activity and protein synthesis during vasopressin-induced cardiac hypertrophy. Xu and Grabowski [2005] reported that acid β-glucosidase mRNA translation is modulated by PKC signaling pathways that are mediated through an 80 kDa mammalian cytoplasmic translational control protein in HepG2 cells. In the present data, we suggest that the MMP-2 regulated by PKC in decidual tissue may be affected by the process of translation.

It is considered that progesterone could transform stromal cells into decidual cells during the decidualization of the endometrium [Tang et al., 1994]. Zhang et al. [2000] reported that progesterone inhibits the activation of MMP-2 in the endometrium. This study found that TPA treatment is able to reverse the inhibition, thus we suggest that it would secrete a hormone to induce the activation of PKCα after the formation of decidual cells by progesterone, and then promote the expression of MMP-2 to enhance the proliferation of decidual cells.

Gonthier et al. [2009] reported that the addition of the PKCα/β inhibitor Gö-6976 is sufficient to abolish the class 3 semaphorins (Sema3A) growth promoting effect and the increase of MMP-2 protein level and activity in cortical dendrites. It is suggested that the selective recruitment and activation of MMP-2 in response to Sema3A requires a PKCα dependent mechanism. In contrast, the PGF2α- and latanoprost-acid-induced MMP-2 secretion and activation in human ciliary muscle cells is blocked by the MEK inhibitor PD-98059, the broad-range PKC inhibitor chelerythrine chloride, and the PKCε translocation inhibitor, but is not blocked by PKCα/β inhibitor Gö-6976, suggesting that prostanoid F receptors activation leads to an increase in the secretion and activation of MMP-2 through PKCα-independent pathways [Husain and Crosson,

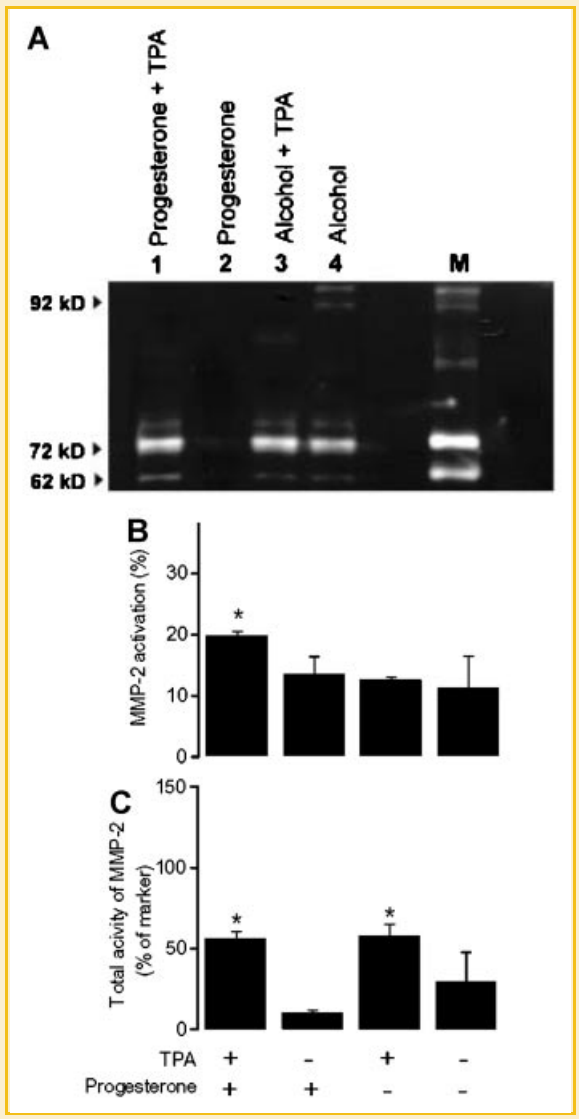

Fig. 7. The effect of TPA (100 ng/ml) on the secretion of MMPs in progesterone (1 μM)-treated decidual cells. A: The samples of the cultured medium were processed for zymography. The indications of 92, 72, and 62 kDa were marked according to previous study. M is the extract of breast cancer tissue and defined as a marker. B: The quantitative data of the activation of MMP-2 were represented. C: The quantitative data of the total activity of MMP-2 were represented. Data are expressed as mean ± SEM for 3–5 measurements. $^{*}P < 0.05$ versus the control of alcohol-treated group.

2008]. However, although the mechanism of MMP-2 activation in decidualization remains discussed, this study demonstrates that MMP-2 is activated and increasingly expressed in early decidualization, and suggests that PKC, especially PKCα, may be involved in the activation and expression of MMP-2 in decidual cells. Moreover, PKCα may regulate the expression of MMP-2 in the translation process.

ACKNOWLEDGMENTS

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REFERENCES

Arslan A, Almazan G, Zingg HH. 1995. Characterization and co-culture of novel nontransformed cell lines derived from rat endometrial epithelium and stroma. In Vitro Cell Dev Biol Anim 31:140–148.

da Rocha AB, Mans DR, Lenz G, Fernandes AK, de Lima C, Monteiro VF, Gonçalves D, Moreira JC, Brunetto AL, Rodnight R, Schwartsmann G. 2000. Protein kinase C-mediated in vitro invasion of human glioma cells through extracellular-signal-regulated kinase and ornithine decarboxylase. Pathobiology 68:113–123.

Das SK, Yano S, Wang J, Edwards DR, Nagase H, Dey SK. 1997. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in the mouse uterus during the peri-implantation period. Dev Genet 21:44–54.

Defeo VJ. 1963. Determination of the sensitive period for the induction of deciduomata in the rat by different inducing procedures. Endocrinology 73:488–497.

Finn CA, Keen PM. 1963. The induction of deciduomata in the rat. J Embryol Exp Morphol 11:673–682.

Freitas S, Meduri G, Le Nestour E, Bausero P, Perrot-Applanat M. 1999. Expression of metalloproteinases and their inhibitors in blood vessels in human endometrium. Biol Reprod 61:1070–1082.

Gamou S, Shimizu N. 1994. Calphostin-C stimulates epidermal growth factor receptor phosphorylation and internalization via light-dependent mechanism. J Cell Physiol 158(1): 151–159.

Goffin F, Munaut C, Frankenne F, Perrier D'Hauterive S, Béliard A, Fridman V, Nervo P, Colige A, Foidart JM. 2003. Expression pattern of metalloproteinases and tissue inhibitors of matrix-metalloproteinases in cycling human endometrium. Biol Reprod 69:976–984.

Golander A, Zakuth V, Shechter Y, Spirer Z. 1981. Suppression of lymphocyte reactivity in vitro by a soluble factor secreted by explants of human decidua. Eur J Immunol 11:849–851.

Goldman S, Shalev E. 2006. Difference in progesterone-receptor isoforms ratio between early and late first-trimester human trophoblast is associated with differential cell invasion and matrix metalloproteinase 2 expression. Biol Reprod 74:13–22.

Gonthier B, Koncina E, Satkauskas S, Perraut M, Roussel G, Aunis D, Kapfhammer JP, Bagnard D. 2009. A PKC-dependent recruitment of MMP-2 controls semaphorin-3A growth-promoting effect in cortical dendrites. PLoS One 4:e5099.

Hashizume K, Takahashi T, Shimizu M, Todoroki J, Shimada A, Hirata M, Sato T, Ito A. 2003. Matrix-metalloproteinases-2 and -9 production in bovine endometrial cell culture. J Reprod Dev 49:45–53.

Hurst PR, Palmay RD. 1999. Matrix metalloproteinases and their endogenous inhibitors during the implantation period in the rat uterus. Reprod Fertil Dev 11:395–402.

Husain S, Crosson CE. 2008. Role of PKCepsilon in PGF2alpha-stimulated MMP-2 secretion from human ciliary muscle cells. J Ocul Pharmacol Ther 24:268–277.

Husain S, Jafri F, Crosson CE. 2005. Acute effects of PGF2alpha on MMP-2 secretion from human ciliary muscle cells: A PKC- and ERK-dependent process. Invest Ophthalmol Vis Sci 46:1706–1713.

Kersten H, Rauen HM. 1961. Degradation of deoxyribonucleic acid in Escherichia coli cells treated with mitomycin C. Nature 190:1195–1196.

Kirk JM. 1960. The mode of action of actinomycin D. Biochim Biophys Acta 42:167–169.

Liu JY, Shyu JC, Chang CL, Tsai CC, Chang AC, Yang LC, Lin LY, Hsieh YS. 1998. Protein kinase C isoforms during the development of deciduomata in pseudopregnant rats. Life Sci 63:721–730.

Matsumoto K, Yamauchi N, Watanabe R, Oozono S, Kubota K, Nishimura K, Wood C, Soh T, Kizaki K, Hattori MA. 2009. In vitro decidualization of rat endometrial stromal cells. Cell Tissue Res 335:575–583.

Mountain DJ, Singh M, Menon B, Singh K. 2007. Interleukin-1beta increases expression and activity of matrix metalloproteinase-2 in cardiac microvascular endothelial cells: Role of PKCalpha/beta1 and MAPKs. Am J Physiol Cell Physiol 292:C867–C875.

Nuttall RK, Kennedy TG. 1999. Gelatinases A and B and tissue inhibitors of metalloproteinases 1, 2 and 3 during in vivo and in vitro decidualization of rat endometrial stromal cells. Biol Reprod 60:471–478.

Obrig TG, Culp WJ, McKeehan WL, Hardesty B. 1971. The mechanism by which cycloheximide and related glutarimide antibiotics inhibit peptide synthesis on reticulocyte ribosomes. J Biol Chem 246:174–181.

Osteen KG, Rodgers WH, Gaire M, Hargrove JT, Gorstein F, Matrisian LM. 1994. Stromal-epithelial interaction mediates steroidal regulation of metalloproteinase expression in human endometrium. Proc Natl Acad Sci USA 91:10129–10133.

Parodi MT, Varesio L, Tonini GP. 1990. The specific inhibitor of protein kinase C, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), induces morphological change and cell differentiation of human neural crest-derived cell lineages. FEBS Lett 269:4–6.

Rotenberg SA, Huang MH, Zhu J, Su L, Riedel H. 1995. Deletion analysis of protein kinase C inactivation by calphostin C. Mol Carcinog 12:42–49.

Salamonsen LA, Nagase H, Suzuki R, Woolley DE. 1993. Production of matrix metalloproteinase 1 (interstitial collagenase) and matrix metalloproteinase 2 (gelatinase A: 72 kDa gelatinase) by ovine endometrial cells in vitro: Different regulation and preferential expression by stromal fibroblasts. J Reprod Fertil 98:583–589.

Sharma A, Masri J, Jo OD, Bernath A, Martin J, Funk A, Gera J. 2007. Protein kinase C regulates internal initiation of translation of the GATA-4 mRNA following vasopressin-induced hypertrophy of cardiac myocytes. J Biol Chem 282:9505–9516.

Shyu JC, Hsieh YS, Chang CL, Tsai CC, Liu HC, Chang AC, Yang LC, Lin LY, Liu JY. 1999. Protein kinase C isoforms during the development of deciduomata in pregnant rats. Life Sci 64:2367–2373.

Spencer F, Chi L, Zhu MX. 1998. Time-dependent relationship between the estrogen receptors and the matrix metalloproteinases following deciduoma induction in rats. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 120:283–288.

Tang B, Guller S, Gurpide E. 1994. Mechanism of human endometrial stromal cells decidualization. Ann NY Acad Sci 734:19–25.

Uhm JH, Dooley NP, Villemure JG, Yong VW. 1996. Glioma invasion in vitro: Regulation by matrix metalloprotease-2 and protein kinase C. Clin Exp Metastasis 14:421–433.

Whiteside EJ, Jackson MM, Herington AC, Edwards DR, Harvey MB. 2001. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-3 are key regulators of extracellular matrix degradation by mouse embryos. Biol Reprod 64:1331–1337.

Wu TT, Tsai JH, Tsai JH, Kuo SJ, Yu SY, Huang CY, Hsieh HY, Hsieh YS, Liu JY. 2004. Augmented release of matrix metalloproteinase-9 by PKC activation in organotypic cultures of human breast cancer and adjacent normal breast tissue and fibroadenoma. Chin J Physiol 47:73–78.

Xu YH, Grabowski GA. 2005. Translation modulation of acid beta-glucosidase in HepG2 cells: Participation of the PKC pathway. Mol Genet Metab 84:252–264.

Zhang J, Hampton AL, Nie G, Salamonsen LA. 2000. Progesterone inhibits activation of latent matrix metalloproteinase (MMP)-2 by membrane-type 1 MMP: Enzymes coordinately expressed in human endometrium. Biol Reprod 62:85–94.

Zhao YG, Xiao AZ, Cao XM, Zhu C. 2002. Expression of matrix metalloproteinase -2, -9 and tissue inhibitors of metalloproteinase -1, -2, -3 mRNAs in rat uterus during early pregnancy. Mol Reprod Dev 62:149–158.