The importance of fibroblasts in remodelling of the human uterine cervix during pregnancy and parturition

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It is well established that fibroblasts play a crucial role in pathophysiological extracellular matrix remodelling. The aim of this project is to elucidate their role in normal physiological remodelling. Specifically, the remodelling of the human cervix during pregnancy, resulting in an enabled passage of the child, is used as the model system. Fibroblast cultures were established from cervices of non-pregnant women, women after 36 weeks of pregnancy and women directly after partus. The cells were immunostained and quantified by western blots for differentiation markers. The cultures were screened for cytokine and metalloproteinase production and characterized by global proteome analysis. The cell cultures established from partal donors differ significantly from those from non-pregnant donors, which is in accordance with in vivo findings. A decrease in α-smooth actin and prolyl-4-hydroxylase and an increase in interleukin (IL)-6, IL-8 and matrix metalloproteinases (MMP)-1 and MMP-3 were observed in cultures from partal donors. 2D-gel electrophoresis followed by mass spectrometry showed that the expression of 59 proteins was changed significantly in cultures of partal donors. The regulated proteins are involved in protein kinase C signalling, Ca2+ binding, cytoskeletal organization, angiogenesis and degradation. Our data suggest that remodelling of the human cervix is orchestrated by fibroblasts, which are activated or recruited by the inflammatory processes occurring during the ripening cascade.

**Key words:** Fibroblast/human uterine cervix/interleukins/proteome/remodelling

**Introduction**

Remodelling of the extracellular matrix (ECM) is a prominent feature during development and normal physiological processes such as wound healing, pregnancy and growth (Westergren-Thorsson et al., 1998; Sennstrom et al., 2000; Quan et al., 2004). An intensive remodelling, resembling that of post-inflammatory processes, also occurs in asthma (Westergren-Thorsson et al., 2002), rheumatic diseases (Westergren-Thorsson et al., 1996; Eklund et al., 2002) and tumour metastasis (Mousa, 2002). In all these cases, highly active fibroblasts produce structural ECM-components, cytokines and ECM-degrading enzymes. In several of these normal remodelling processes, as well as in diseased states, recruitment of blood-borne fibroblast precursors (fibrocytes) occurs (Quan et al., 2004).

Fibroblasts in the remodelling process are regulated by cytokines, both in autocrine and paracrine fashion. The cytokines are produced by activated fibroblast, macrophages, neutrophils and lymphocytes. Examples of regulatory cytokines in these processes are transforming growth factor-β (TGF-β), epidermal growth factor, platelet-derived growth factor and various interleukins (ILs), where TGF-β is known as the main enhancer of ECM production with effects on both synthesis and the final structure of the products (Westergren-Thorsson et al., 1992). The effects of cytokines on fibroblasts, however, differ considerably depending on tissue and localization within the tissue (Westergren-Thorsson et al., 1990). Furthermore, several fibroblast clones, with distinctly different properties, are present in normal, persisting tissues and tissues undergoing remodelling (Westergren-Thorsson et al., 2004).

Extensive tissue remodelling occurs during pregnancy. The uterus undergoes an anabolic process where both smooth muscle and connective tissue increases (Hjelm et al., 2001a, b). The cervix, on the other hand, which is mainly composed of fibrous connective tissue, is remodelled in a two-step process. During the first 36 weeks, a hormone-driven decrease in collagen and proteoglycans (Uldbjerg et al., 1983a) dominates. At the onset of labour, the cervix needs to be a soft and elastic organ and crucial for the passage of the foetus. This is obtained by a breakdown and reconstitution of the ECM, achieved via recruitment of neutrophils (Stygar et al., 2002), an increase in metalloproteinases (MMPs) (Sennstrom et al., 2003) and changed ECM production (Westergren-Thorsson et al., 1998).
During this final ripening step, cytokines such as IL-6, IL-8 and G-CSF increase at least a 100-fold (Sennstrom et al., 2000), which has major effects on ECM production. IL-8 promotes recruitment and activation of neutrophils, which in turn stimulate IL-6 and IL-8 production from fibroblasts (King et al., 2001). The activated neutrophils secrete proteolytic enzymes, such as MMP-8 and leukocyte elastase (Sennstrom et al., 2003), necessary for the final breakdown of the collagen network. After partus, a rapid restoration of cervix to an ‘unripe state’ occurs (Westergren-Thorsson et al., 1998).

Different cells are involved in this remodelling process, but several studies indicate that fibroblasts are key players in the final stage by production of ILs, MMPs and ECM components such as the versican proteoglycans (Sennstrom et al., 1997; Westergren-Thorsson et al., 1998; Sennstrom et al., 2000; Sennstrom et al., 2003). In this study, we established fibroblast cultures from cervices of women that were non-pregnant, 36-week pregnant or partal to test whether the hypothesis that fibroblasts having different properties are involved in the cervical remodelling process is valid. Fibroblasts from 36-week pregnant or partal women differed markedly form their non-pregnant counterparts in terms of immunohistological markers, cytokine and MMP production and also had a dramatically changed proteome. We conclude that the specific fibroblasts are activated or recruited during the ripening process and are necessary to fulfill the final cervical remodelling occurring only hours prior to birth.

Materials and methods

Chemicals
Monoclonal antibodies against prolyl-4-hydroxylase (#M0877) and α-smooth actin (α-SMA) (#M0851) and the alkaline-phosphatase-conjugated secondary rabbit anti-mouse antibody (#D0314) were purchased from DakoCytomation, Denmark. The monoclonal antibodies against IL-8 (NAP-1) came from Miroslav R. Vienna, Austria, and biotinylated goat anti-mouse immunoglobulin G (IgG) was purchased from Caltag Laboratory, San Francisco, USA. The secondary horse anti-mouse (IgG), the avidin–biotin detection system Vectastain ABC-elite and 3,3′-diaminobenzidine (DAB) were purchased from Vector Laboratories, Burlingame, USA. The goat anti-mouse secondary antibody, Alexa FluorTM 594, was purchased from Molecular Probes, The Netherlands. 3-Amino-9-ethyl-l-carbazole (AEC) came from Sigma Chemicals Co, St Louis, USA. The Immulite® Immunoassay system was from Diagnostic Products Corporation, Los Angeles, USA. The sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on NuPAGE® 4–12% Bis Tris gradient gels (#NP3026BOX) from Invitrogen Life Technologies, USA. Cell culture materials, including antibiotics, amino acids and cell culture medium, were purchased from Gibco, Sweden. All other chemicals were of analytical grade.

Tissue collection
Cervical biopsies (50–300 mg) were obtained transvaginally from the distal part of the human cervix from pregnant and non-pregnant women. Biopsies were taken at elective caesarean sections from term (>36 + 6 weeks)-pregnant women not in labour and with unripe cervixes (n = 5) and from women at spontaneous vaginal partus (n = 5). Non-pregnant cervical tissue samples obtained from fertile women, undergoing hysterectomy due to benign disorders such as myomas or menorrhagia, served as controls (n = 5). The Ethics Committee at the Karolinska Hospital, Solna, Sweden, approved the study, and all biopsies were obtained with informed consent.

Cell culture
The biopsies were washed in Earle’s minimal essential medium (EMEM) and cut into ~1-mm³ pieces. These were spread on the bottom of growth flasks and allowed to adhere, and EMEM with penicillin (1%), streptomycin (1%), glutamine (1%) and calf serum (10%) was added. Cells were grown until the isolated fibroblast populations were confluent and transferred to new culture flasks three to four times prior to the experiments. Mycoplasma infections were excluded using 4,6-diamidino-2-phenyl-dihydrochloride. The cell cultures were studied between passages 4 and 8.

Immunohistochemistry
For an α-SMA detection, biopsies were fixed for 12–24 h in 4% paraformaldehyde, kept in ethanol (70%) until embedded in paraffin and then sectioned at 5 μm. After de-paraffinization with xylene and rehydration with ethanol, a monoclonal mouse anti-human α-SMA antibody (dilution 1 : 200) was added to the sections. For detection of prolyl-4-hydroxylase, samples were cryosectioned in 8-μm sections and fixed in acetone for 3 min and acetone:methanol (1 : 1) before adding a monoclonal mouse anti-human prolyl-4-hydroxylase antibody (dilution 1 : 50). Horse anti-mouse IgG (dilution 1 : 200) was used as secondary antibody, both for α-SMA and prolyl-4-hydroxylase. For detection of IL-8 expression, biopsies were treated as described previously (Sennstrom et al., 2000). In short, the biopsies were sectioned and fixed in 2% paraformaldehyde. Saponin was used to permeabilize the cell membranes. The sections were incubated overnight at room temperature in a humidified chamber with the monoclonal antibodies for IL-8 (NAP-1). A biotinylated goat anti-mouse IgG antibody was used as secondary antibody. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxidase in methanol. Non-specific binding was blocked with 10% horse serum. After staining with the primary and secondary antibodies the slides were incubated with an avidin–biotin–peroxidase complex and developed with either AEC for red staining (prolyl-4-hydroxylase) or DAB for brown staining (α-SMA and IL-8). The slides were washed in water, counter stained with 10% Mayer’s haematoxylin for 3 min and then mounted. Two investigators evaluated the staining independently using a Zeiss Axioskop. Photos were taken with a digital colour video camera (Sony, Exwvave HAD). Negative controls were treated in an identical manner, except that the primary antibody was omitted. Staining specificity was tested by incubation with recombinant produced proteins. Placenta (IL-8) and uterine tissue were used as positive controls.

Cytokines in fibroblast cultures
Levels of ILs-6 and -8 were analysed in medium using a solid-phase, two-site chemiluminescent enzyme immunometric assay with the IMMULITE AutoAnalyzer, as described earlier (Berthier et al., 1999). For IL-8, the intra-assay variation was calculated to 3.6–3.8%, inter-assay variation 5.2–7.4% and sensitivity 2 pg ml⁻¹. There was no cross-reactivity with IL-1β, IL-2, IL-2R, IL-4, IL-6, interferons or TNF-α and no interference with EDTA or heparin. For IL-6, the intra-assay variation was 3.0–8.4%, inter-assay variation 6.2–9.7% and sensitivity 1 pg ml⁻¹. There was no cross-reactivity with IL-2, IL-2R, IL-8, IL-1β and TNF-α and no interference with EDTA.
MMP production in fibroblasts cultures
The MMPs were analysed in medium from fibroblasts by immunoassay 24 h after fresh medium was added. For MMP-1, this assay determines total MMP-1 (i.e. free MMP-1 and MMP-1 in complex with tissue inhibitor of metalloproteinase-1 (TIMP-1), but not MMP-1 bound to α-2-macroglobulin). According to the manufacturer, there is no cross-reactivity with α-2-macroglobulin. The MMP-3 assay measures total MMP-3, including proMMP-3, active MMP-3 and MMP-3/TIMP complexes, but not MMP-3 bound to α-2-macroglobulin. There is no cross-reactivity with MMP-1, MMP-2 or MMP-9 or TIMP-1 or TIMP-2, according to the manufacturer.

The detection limit was 6.25 ng ml$^{-1}$ for MMP-1 and 3.75 ng ml$^{-1}$ for MMP-3. Standard MMP-1 and MMP-3 were purchased from Amersham, Bucks, UK. The assay was performed according to the instructions of the manufacturer, and all samples were assayed in duplicates. The results are expressed as percent of non-pregnant control and the median levels in the groups are expressed as nanogram per millilitre medium.

Expression profiles of abundant proteins by 2-Dgel electrophoresis
The cervical fibroblasts were cultured in six-well plates ($6 \times 8.3$ cm$^2$) and were, at confluence, rinsed twice with ice-cold PBS and scapped into 200 µl of 7 M urea, 2 M thiourea and 4% CHAPS as described previously (Malmstrom et al., 2002a). The protein extracts were precipitated with four volumes of ice-cold acetone and re-dissolved in scraping buffer. About 150 µl of the cell extract was mixed with 200 µl of solubilization solution containing 7 M thiourea, 2 M urea, 4% CHAPS, 10 mM DTT and 0.5% IPG 4-7 buffer and added to Immobiline Dry strips (180 mm, pH 4-7 NL) (Amersham Pharmacia Biotech, Uppsala, Sweden) for overnight dehydration. The isoelectric focusing step was performed at 20°C in a Multiphor™ (Amersham Pharmacia Biotech) and run according to the following schedule: 0-300 V for 1 h, 300-3500 V for 1.5 h and finally 3500 V for 23 h, until 83 kV h$^{-1}$ was reached. The strips were reduced for 10 min in 65 mM DTT, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS and 50 mM Tris–HCl, pH 8.8, and alkylated for 10 min in the same solution except for DTT, which was replaced with 0.25 M iodoacetamide. The strips were soaked in electrophoresis running buffer (24 mM Tris base, 0.2 M glycine and 0.1% SDS) just before the 2D-gel electrophoresis and the strips were applied on 14% homogeneous Duracryl slab gels. The strips were overlaid with a solution of 1% agarose in electrophoresis buffer (kept at 60°C) and electrophoresis was carried out in a Hoefer™ DALT gel apparatus (Amersham Pharmacia Biotech) at 20°C with constant voltage (100 V) for 18 h. The gels were stained with a silver solution according to a protocol described by Shevchenko et al. (1996) and scanned using a GS-710 Calibrated Imaging Densitometer (Bio-Rad Laboratories, Sundberg, Sweden). Spot analysis was performed using the PDQUEST (version 7.0) 2D-gel analysis system (Bio-Rad discovery series, Bio-Rad Laboratories). Spots with a significant Wilcoxon P-value were validated by visual inspection using a gel-slicing feature of the 2DDB software. Every spot on all the gels were assigned an integrated optical density (IOD) value by the software program. This value was divided by the total IOD for all detected spots. Thus, each spot is expressed as parts per million of the total IOD of all valid spots. The gels were run in triplicate, thus each experiment comprised nine gels (n = 3, i.e. 27 gels). Match sets containing 9 gels and 27 gels were made and analysed separately as a control of the analysis schema. After matching, the data were exported to an SQL relational database, where the individual experiments were further matched by x,y co ordinates and statistical analysis was performed. Spots with a P-value <0.05 were sliced out and transferred to small Eppendorf tubes, followed by washing (40% acetonitrile, 60 mM ammonium bicarbonate, pH 7.8). The gel plugs were dried down in a Speed Vac for 15 min (Savant Speed Vac, DNA 110) and digested with trypsin (Promega Porcine, acetic acid) in 25 mM ammonium bicarbonate and incubated overnight at 37°C. The digestions were stopped by adding 0.2% trifluoroacetic acid.

Mass-spectrometry identification
Ziptips (Millipore, Bedford, MA, USA) were used to concentrate and desalt the protein digests according to the manufacturer’s instructions. After washing, the peptides were eluted on an Anchor-chip™ plate (Bruker Daltonics, Bremen, Germany) and mixed with 0.5 µl of 2.5–dihydroxybenzoic acid matrix (2.8 mg ml$^{-1}$). The matrix-assisted laser desorption ionization time of flight (MALDI-TOF) instrument used was a Bruker REFLEX (Bruker-Daltonics) operated in a reflector mode at an accelerating voltage of 20 kV. The data were analysed using both the Swissprot and NCBI database using Profound (http://prowl.rochelever.edu/profound_bin/WebProFound.exe).

Statistical methods
Spot data and gel images were exported from PDQUEST and imported into an in-house-developed analysis software (Malmstrom et al., 2002b). In short, the software integrates PDQUEST data with bioinformatical and statistical tools. PDQUEST-matched spots from the same treatment were compared with PDQUEST-matched spots from the two other treatments using Wilcoxon signed-rank test. Spots with a quality score below 25 and quantity score below 100 were omitted. Because of the relative measure (IOD), the spot intensities were normalized to non-pregnant controls. Identified proteins were, when possible, assigned GO terms, either from the proteins themselves or from close orthologues. For IL-6, IL-8, MMP-1 and MMP-3 analyses, the Mann–Whitney U and Kruskal–Wallis tests were used to estimate P-values.

Results
Expression of fibroblast markers in cervical tissue and fibroblasts cultures
The human cervix undergoes an extensive remodelling during pregnancy and at parturition. During the final stages of pregnancy, a weak decrease in prolyl-4-hydroxylase staining is observed, which is in agreement with the substantial decrease of collagen concentration observed at term and in partial cervixes (Figure 1). Since it is seen in tissues from both 36-week-pregnant and partial women, changed collagen turnover is a feature of the slower, more long-lasting phase of the cervical remodelling. Staining for α-SMA, a marker for myofibroblast differentiation, is, however, not clearly decreased until the very last phase (Figure 1). This may indicate that contractile elements in the fibroblasts of cervix are needed to retain the fetus in the uterus until the delivery process is initiated. To investigate whether these changes in expression patterns also occur in fibroblast cultures from the different stages of pregnancy, we established several cultures from each state. The expressions of α-SMA (Figure 2A–D) and prolyl-4-hydroxylase (Figure 3A–D) were then studied by western-blot analysis and immunohistochemistry. The data were in accordance with the tissue sections, with an ~70% decrease in the prolyl-4-hydroxylase expression in fibroblasts from both 36-week-pregnant and partial women, whereas α-SMA expression was only significantly decreased in partial fibroblast (40% of control).

Expression of inflammatory markers in cervical tissue and fibroblasts cultures
In vivo, the amounts of IL-6 and IL-8 (Sennstrom et al., 2000) increase 100–1000-fold during the late phase of pregnancy. It was therefore of interest to examine the production of these cytokines in corresponding cervical tissues and fibroblast cultures. Immunohistochemistry revealed an elevated expression of IL-8 term-pregnant material, obtained at 36 weeks of pregnancy, and an even stronger expression in partial tissue (Figure 1). This staining is found close to both fibroblasts and neutrophils, especially in partial tissue, but their site of synthesis, is unclear. To further collect information of sites of synthesis, we studied whether the fibroblasts in cultures established from non-pregnant, term pregnant and partial tissues produce IL-6 and IL-8, using the IMMUNOLITE methodology. IL-6 showed a significant increase in partial samples. In comparison with non-pregnant cultures, a mean 2.5-fold increase in cultures from term-pregnant patients was seen, however not statistically significant. In partial cultures, a significant 3.5-fold increase was recorded (Figure 4, $P = 0.0433$). The same pattern was noted for IL-8, where term-pregnant donors increased on an average 3.5-fold compared with controls, whereas those from partial donors increased significantly 5–6-fold (Figure 4, $P = 0.0209$).
The high expression of cytokines was noted until passage eight and therefore constitutes a true phenotype of the cells and is not a paracrine phenomenon because of the contamination of other cell types. Thus, the different expressions of α-SMA, prolyl-4-hydroxylase, IL-6 and IL-8 demonstrate the presence of different types of fibroblasts during the process of cervical ripening.

**MMPs increase in cultures from pregnant donors**

Earlier work has demonstrated that MMP-8, derived from recruited neutrophils, is an important player in the final remodelling process, but increase in MMP-1 and MMP-3 are also observed in the cervical matrix (Sennstrom et al., 2003). It was therefore of interest to...
investigate whether the cervical cells secrete these MMPs. Indeed, cultures from non-pregnant donors did secrete an appreciable amount of both MMP-1 and MMP-3 (Figure 5). However, cultures from term-pregnant donors showed 40–70% higher mean secretion and cultures from partal donors showed an even higher secretion, with a 2–3-fold increase in MMP-1 and MMP-3 secretion, respectively (Figure 5, $P = 0.0275$). This feature was also stable in eight passages, further implying that it is a true endogenous phenotype of the cells, not exogenously regulated.

**Proteome analysis**

2D-gel electrophoresis, analysed by PDQUEST and MALDI-TOF, was used to study the protein expression pattern in the cell cultures established from the non-pregnant, 36-week-pregnant and partal subjects. A typical gel, with the $pI$ range from 4 to 7, is shown in Figure 6. Using cell cultures from a total of nine patients and performing 2D-gel sets in triplicates enabled us to establish a hierarchical gel set with, in total, 519 matched spots. Forty-four of these were assigned an identity. The IOD of the spots were normalized against the non-pregnant group, and a total of 22 significantly regulated components were detected in partal cultures. In cultures from 36-week-pregnant women, 16 significantly regulated spots were found, compared with cultures from non-pregnant donors. Four regulations were noted when cultures from term-pregnant and partal donors were compared. Of the regulated components, 12 were identified, and of these, 11 were up-regulated up to 4–5-fold. Only one protein was down-regulated by $\sim 50\%$ in partal cultures (Table I). The identified proteins were separated into groups reflecting the function of the proteins (Table I). Vimentin, which is involved in cytoskeletal organization and related signalling and is regarded as a marker for fibroblasts, is up-regulated 2–3-fold (Figure 6). Of note is the increased expression of proteins of the 14-3-3 family, where especially 14-3-3 protein theta influencing Protein kinase C (PKC)-activity is significantly up-regulated around 5-fold in both term and partal cultures (Figure 6). Also Ca$^{2+}$-binding proteins such as annexin V are clearly induced (Table I). On the other hand, calreticulin, which is a chaperon taking part in glycoprotein quality control/folding, is strongly repressed (Table I). The protease cathepsin B is also up-regulated, which probably is of importance for final ECM degradation (Figure 6).

Associated with the cervix involution is the increased expression of angiogenin inhibitor, which is only induced in the partal cultures (Figure 6). This indicates that these proteins play a role in the final remodelling, where a decrease in the vascular bed and formation of ECM are hallmarks (Table I).
Discussion

ECM remodelling occurs both under normal conditions and during many pathological processes. In this study, we have followed cells established from different stages of remodelling of the human uterine cervix during pregnancy and parturition. Our data indicate that activated fibroblasts are important players in this remodelling and that several types of phenotypically different fibroblasts appear in the cervical tissue throughout the process. The main feature of the cervical ripening is the gradual change of a stiff, ECM-dominated organ into a soft, permissible structure, allowing the passage of the child. It occurs in two phases: an estrogen/progesterone driven one, leading to a term-pregnant cervix (Uldbjerg et al., 1983a), and a rapid phase, immediately prior to birth, with inflammation-like remodelling features. In the first stage, there is a slow remodelling, executed by fibroblasts, which by changing the turnover rate, decrease ECM components (Norman et al., 1991). At partus a different, very active remodelling takes place, mediated through high concentrations of PGE2, NO, MMP-1, MMP-3, G-CSF, IL-6 and IL-8, where the latter two recruit and activate neutrophils (Sennstrom et al., 2000). Our data are however in contrast to those of Sakamoto et al. (2004, 2005), who do not find any correlation of cervical ripening and interleukin expression. We clearly find significant increases of interleukins at term pregnancy. These differences may be due to the selection of term-pregnant patients, which is a highly variable group regarding cervical ripening. To determine whether the observed changes are due to a final remodelling or initial involution, further studies must be performed.

Cell cultures established from different stages of ripening are phenotypically different and express properties typical of the in vivo situation they are derived from. In tissues of non-pregnant patients, as well as in cultures thereof, high collagen and proteoglycan production (mainly decorin) (Uldbjerg et al., 1983b) and a high α-SMA expression are found (Figures 1 and 2). This suggests the importance of a dense, fibrous matrix in the non-pregnant cervix. In tissue from term-pregnant and partal patients, and cultures thereof, there is, on the contrary, a high expression of IL-6, IL-8, MMP-1 and MMP-3, whereas collagen and α-SMA content are lowered. This reflects the necessity of a different set of cells to promote ECM degradation in the preparation of a successful delivery. When this tightly regulated process is at fault, pre-term delivery may occur, at great danger to the unborn fetus. Interestingly, the cell cultures are phenotypically stable for at least eight passages in culture, showing that the differences are not due to any extrinsic cytokines or hormonal influence. Rather recruitment, or differentiation from progenitors on site to activated inflammatory fibroblasts cells, may occur. Furthermore, the proteome of cultures from non-pregnant and partal donors differs clearly in protein expression, where several differently regulated components were detected. Twelve of these proteins were identified and belong to proteins involved in cytoskeletal functions, signalling, Ca²⁺ binding, degradation and involution. Cultures from term-pregnant donors showed only two identified significant regulations, compared with either the non-pregnant or the partial states. This can be explained by the variable state of these patients, since they are at different stages of remodelling at this time of pregnancy. Unfortunately,
proteomic analyses using 2D gels have shortcomings concerning low-expressed proteins as well as extensively modified proteins. Thus, using this system, we cannot detect the low-expressed ILs and matrix components as collagens and proteoglycans.

Of great interest is the 5-fold up-regulation of a group of PKC activator theta proteins (Table I). An increase in these activators could potentially activate several signal transduction pathways, together with Ca\(^{2+}\) and/or diacylglycerol. This results in modification of activity of various groups of PKCs, which contain at least 11 subgroups. The downstream signalling involves mitogen-activated protein kinase (MAPK) (Ueda et al., 1996) and further c-fos and c-Jun, activating AP-1 sites in promoter sequences. Possible downstream targets of this pathway are the genes of MMP-1 and MMP-3, which are increased 2–3-fold in partial cells. The regulation of MMP-1 and MMP-3 expressions has earlier been shown to independently rely on both the PKC and the p44/42 MAPK pathways (Reuben et al., 2002). Furthermore, the activation of PKC induces collagenase activity in the cervix of pregnant guinea pigs (Rajabi et al., 1992), implying its importance in the signal transduction occurring during cervical ripening.

Up-regulation of IL-8 and IL-6, >100-fold in the human cervix in vivo, has also been recorded during cervical ripening (Sennstrom et al., 2000). The results from the present study show that the same phenomenon occurs in fibroblast cultures, however, more pronounced in partial cultures compared with term-pregnant cultures. One possible pathway is induction of IL-8 by increased Ca\(^{2+}\) flux (Marino et al., 2003), followed by activation of PKC and NF-κB (Cummings et al., 2004; Page et al., 2003). Induced changes of c-fos and c-Jun influence the AP-1 site, which is found in the IL-8 promoter region (Cummings et al., 2004). The up-regulation of activators of PKC suggests that it is involved in signalling leading to IL-8 production, both in vivo and in the term-pregnant and partial cell cultures. Also, IL-6 production is stimulated by increased Ca\(^{2+}\) (Jeong et al., 2002, Giugliano et al., 2003) and ERK and PKC activation (Graness et al., 2002). Thus, the up-regulation of PKC activators and a changed Ca\(^{2+}\)-sensitivity could be one mechanism for IL-6 secretion in partial cells.

During involution after partus, a remodelling back to the non-pregnant state occurs rapidly (Westergren-Thorsson et al., 1998) and is featured by a significant increase in collagen and proteoglycan production. It is therefore likely that constitutive fibroblasts are re-activated to produce matrix reconstructing the cervical tissue. The fate of the inflammatory fibroblasts after partus is uncertain, but one possibility is that they undergo apoptosis. Furthermore, a hallmark of ripening is a highly increased level of vessels that disappear after parturition. Of interest is therefore the observation that the partial fibroblasts produce a 2-fold higher amount of the angiogenin inhibitor (Table I), preparing the tissue for dilatation and involution.
Two major conclusions can be drawn from these data. The first is the fact that fibroblasts are important cells in tissue remodelling and are involved both in tissue degradation/remodelling and in attraction of neutrophils. The second, that to accomplish this remodelling, fibroblasts with different properties must be engaged. An important question is how these fibroblasts, having inflammatory characteristics, are recruited or activated and how they maintain their activity in vitro. During remodelling of other tissues, for instance, during cancer-induced remodelling, progenitor fibroblasts (fibrocytes) are recruited from the circulation and can differentiate into inflammatory fibroblasts (Abe et al., 2001; Barth et al., 2002). It is also well documented that recruitment and differentiation into active fibroblasts occur in the lungs during fibrosis associated with asthma, scleroderma and interstitial lung fibrosis (Schmidt et al., 2001; Barth et al., 2004). The inflammatory fibroblasts found in the uterine cervix do, however, not generate fibrosis as observed in asthma and lung fibrosis. Another possibility is recruitment of mesodermal progenitors (Pittenger et al., 1999). The results from this study do not provide evidence that recruitment of such cells occurs but the presence of TGF-β in partial cervix (Westergren-Thorsson et al., 1998) suggests that a mesenchymal stem-cell differentiation may be involved (Roelen and Dijke, 2003). TGF-β however also promotes myofibroblast differentiation, which is not the case in this study, where the expression of α-SMA was decreased in partial tissues and cultures.

Cervical remodelling shows similarities to an inflammation-driven remodelling with the presence of ‘inflammatory’ fibroblast and, in the final stage, activated neutrophils. This recruitment of neutrophils is most likely due to the production of ILs-6 and -8, both known attractants of neutrophils (Sennstrom et al., 2000). The key question is what factors induce the very large production of these ILs. Using immunohistochemistry, we have previously shown that IL-8 production initially occurs in the cervical epithelium and that it temporally correlates to fetal fibronectin production from the fetus at term (Ekman et al., 1995). This indicates that other factors, e.g. from fetal tissues, may be involved in recruitment and activation of fibroblasts.

In conclusion, we have shown that fibroblasts are important in cervical tissue remodelling and that different, phenotypically stable, cell clones are found. The activation pathways involved could be Ca2+- and PKC-dependent and result in a considerable increase in IL-6, IL-8, MMP-1 and MMP-3. This is of absolute necessity for a normal softening of the cervical tissue, allowing the dilation and expulsion of the fetus at parturition.

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