REGULATION OF COLLAGENASE IN RABBIT UTERINE CERVICAL FIBROBLASTS

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It is well known that marked decrease in collagen concentration was observed in the uterine cervix during ripening and dilation at term pregnancy (1-4). In addition, collagenase is thought to be the key enzyme to induce the above changes (4-9). Regulation of collagenase in the cervix is not fully clarified until now. We have, therefore, examined the regulation of collagenase activity in the cervix at term pregnancy using the rabbit uterine cervical cells in culture.

I. Production of procollagenase and procollagenase activator by rabbit uterine cervical cells.

A typical mammalian collagenase could be extracted from the homogenates of human and rabbit uterine cervices (5, 6, 11). It is also a fact that obvious collagenolysis was morphologically observed around polymorphonuclear leucocytes exuded into the human cervical tissue at term pregnancy (11). The origin of the cervical collagenase, however, could not be identified until now.

When cervical cells were harvested from rabbits with 23 days of gestational age and maintained in culture of MEM/10% FCS, cervical fibroblast-like cells produced and secreted much collagenase and neutral proteinase as proenzymes into culture medium as shown in Fig. 1. By a chromatography of the culture medium on CM-52 cellulose, non-adsorbed neutral proteinase was separated.

Fig. 1. Synthesis of collagenase and neutral proteinase by rabbit uterine cervical cells in primary culture.

The cells from a 23-days pregnant rabbit were cultured and the medium was harvested every two days. On day 10, the cultures were divided into two groups. One group was control and the other was treated with cycloheximide (7 μg/ml). Collagenase and gelatinolytic activity in the media were assayed after activation with trypsin. (●), collagenase; (○), neutral proteinase; ----, control culture; -----, cycloheximide treatment.
completely from procollagenase bound to the column, and both enzymes were further purified to be electrophoretically single protein by chromatographic procedures. Proproteinase (Mr 60,000) was activated by trypsin, plasmin and 4-aminophenylmercuric acetate (APMA) and the active species of the proteinase had Mr 53,000 when activated by APMA. The purified procollagenase had Mr 55,000 and was activated by trypsin, APMA and the active neutral proteinase. These activation were accompanied by decrease in Mr, and the active species had an Mr which was approx. 10,000 less than that of the procollagenase. In particular, procollagenase activation with neutral proteinase depended on incubation time and proteolytic activity of the proteinase. These results indicate that activation of procollagenase by the rabbit uterine proteinase related to limited proteolysis in the procollagenase molecule is different from that observed with rat uterus (13) and rabbit synovial cells (14). In conclusion, rabbit uterine cervical cells could produce much procollagenase and neutral proproteinase, and the collagenase increased in the ripened uterine cervix is originated from the cervical cells.

2. What is the collagenase regulator in the cervix?

To clarify the regulator of collagenase production, dehydroepiandrosterone sulfate (DHAS) may be one of clues, since DHAS is produced by fetal adrenal gland and changed into estrogens in placenta and significantly high level of DHAS than that of its metabolites was present in the human cervix at term pregnancy (15). In addition, DHAS is clinically used as an accelerator for cervical ripening and dilation. DHAS stimulated the production of both procollagenase and neutral proproteinase, not affecting the cell number, (3H)thymidine incorporation into DNA and protein synthesis of cells (16, 17). Nevertheless, the addition of DHA and estradiol (E₂) to the culture medium caused no significant effect on these enzymes as shown in Fig. 2. Furthermore, we have confirmed that E₂ and progesterone increase significantly the production of collagenase inhibitor without the proliferation of cervical cells (18). We have also reported that receptor-like DHAS-binding protein is present in rabbit and human uterine cervixes and human amnion (19, 20), and that the content of this protein in rabbit uterine cervix increased with the progress of pregnancy.

These results suggested that the effects of DHAS on the cervical ripening, especially, on the collagenase activity are due to unchanged DHAS and that the action of DHAS is mediated via cytoplasmic DHAS-receptor like protein.

It is morphologically observed that numerous leucocytes such as polymorphonuclear leucocytes (PMNL) and macrophages (Mφ) effused into the ripened cervixes. Whereas their functions during cervical ripening were not fully understood. When the conditioned medium of rabbit peritoneal Mφ (MCM) was added to the culture medium of rabbit uterine cervical cell, production of both procollagenase and procollagenase activator was significantly accelerated in a dose dependent manner. This enhancement of collagenase production was depressed by the treatment of cells with cycloheximide, indicating that the action of MCM towards the collagenase production was due to the acceleration of synthesis of these enzymes.

It was found that the collagenase stimulating activity and lymphocytes activating activity were effused in the same fractions by the gel-filtration on Sephadex G-75. These results suggested that uterine cervical cells have sensitivity towards interleukin-1 (IL-1) and such cytokine regulates at least partly collagenase production in the cell.

To clarify the role of IL-1 during the cervical ripening, in vivo, IL-1
Fig. 2. Effect of DHAS, DHA and E₂ on collagenase production by rabbit uterine cervical cells in primary culture.

Primary rabbit uterine cervical cells from 23 days pregnant rabbits were grown to confluent in MEM/10 % (v/v) FCS. Confluent cultures were incubated in the absence (control) or presence of the indicated concen. of the steroids in MEM/10 % (v/v), and the medium harvested on the 4th day after confluence was assayed for collagenase and gelatinolytic activity after activation with trypsin. Data are shown as the mean ± SD of 3 wells. ** and ***; significantly different from control (p<0.01 and p<0.001).

Fig. 3. Gel filtration of IL-1 activity in culture media of rabbit uterine cervix on Sephadex G-75.

Cervical tissue at 30 days gestation and nonpregnant cervix were cultured in DMEM for 7 days and medium was harvested every day. Each pooled medium of 1 g tissue was dialized, lyophilized and then applied to a Sephadex G-75 column (1.3 x 57 cm) previously equilibrated with PBS(-). Fractions were tested in the IL-1 assay at 1:10 dilution. (o), pregnant at 30 days; (●), nonpregnant.
activity in the cervical tissue was examined. Tissue culture media of cervices from rabbits with 30 day gestational age and nonpregnant ones were dialyzed, concentrated and chromatographed on Sephadex G-75. As shown in Fig. 3, the medium of pregnant cervix was found to contain three species with IL-1 activity and collagenase stimulating activity, whereas the media from nonpregnant cervices contained scarcely IL-1 activity. These results indicated that IL-1 is a promotive effector for the cervical ripening, especially via acceleration of collagenase production. At present, however, we did not identify the origin of this tissue IL-1, since IL-1 activity prepared from rabbit peritoneal PMNL and MØ was not distinguished from the tissue IL-1 by the gel-filtration.

Finally, endogeneous IL-1 inducer in the cervix was examined. Since increase in hyaluronic acid in the human and rabbit cervices is generally recognized, hyaluronic acid was added to the rabbit peritoneal MØ culture. Hyaluronic acid stimulates the cells to induce IL-1, and the action of hyaluronic acid similarly exerted to human peripheral monocytes and PMNL, indicating that hyaluronic acid might act as an endogeneous IL-1 inducer in addition to its role for tissue water container during the cervical ripening and dilation (21).

In conclusion, regulation of collagenase in the uterine cervix was summarized as shown in Fig. 4. Uterine cervical cells have a ability to induce procollagenase and procollagenase activator, and DHAS and IL-1 stimulate the production of these enzymes. E₂ and progesterone may inhibit the collagenolysis in the cervix, because these hormones accelerate the production of collagenase.

![Fig. 4. Regulation of collagenase in uterine cervical cells.](image-url)
inhibitor. It is also an interesting fact that hyaluronic acid, one of the connective tissue components in the cervix, acts as an endogenous inducer of IL-1. However, most effective promoter for collagenase production is still unclear.

REFERENCES