with the highest levels at 21 h after RU486 administration. Furthermore, a single injection of progesterone (120 mg/kg) at 2 h before RU486 administration delayed the initiation of RU486-induced preterm birth. These results suggest that the interstitial collagenase activity in the uterine cervix which might be negatively controlled by progesterone plays a significant role in the regulation of the uterine cervical ripening and the maintenance of pregnancy to term in late pregnancy of rats.

ACTIVATION OF THE PROGELATINASE A (PROMMP-2) COMPLEXED WITH TISSUE INHIBITOR OF METALLOPROTEINASE (TIMP)-2 BY A CELL SURFACE ACTIVATOR OF HUMAN UTERINE CERVICAL FIBROBLASTS

Yoshifumi Itoh, Sharon Binner and Hideaki Nagase

Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160-7421, USA

Human uterine cervical fibroblasts in culture constitutively synthesize and secrete matrix metalloproteinase 2 (MMP-2/gelatinase A) as inactive zymogen (proMMP-2). When activated in vitro, MMP-2 is capable of degrading various extracellular matrix components including proteoglycans, fibronectin, elastin, laminin and collagen types IV, V, VII and XI. However, the involvement of MMP-2 in the accelerated tissue matrix catabolism is not clear, because, unlike MMP-1 (interstitial collagenase) or MMP-3 (stromelysin 1), the synthesis of proMMP-2 by the cervical fibroblasts is not altered when the cells are treated with cytokines such as interleukin 1 and tumor necrosis factor α. We therefore postulate the key step regulating MMP-2 activity in the tissue may lie on the activation step but not the production from the cells. The ability of proMMP-2 to form a complex with an endogenous inhibitor TIMP-2 through interaction of the C-terminal domain of each molecule has indicated additional complexity in activation of proMMP-2. In this study, we examined how proMMP-2 complexed with TIMP-2 can be activated as a part of the control mechanisms of MMP-2 activity in the extracellular milieu.

ProMMP-2 and the proMMP-2-TIMP-2 complex were isolated from the culture medium of human cervical fibroblasts by gelatin-Sepharose affinity chromatography and gel filtration on Sephacryl S-200 chromatography. Both proMMP-2 and the proMMP-2-TIMP-2 complex were subjected to activation by 4-aminophenylmercuric acetate (APMA) and by an endogenous membrane bound activator (MbAct) isolated from human cervical fibroblasts treated with concanavalin A.

Incubation of proMMP-2 with either APMA or MbAct at 37°C activated the zymogen and generated the 67-kDa MMP-2. However, treatment of the proMMP-2-TIMP-2 complex with APMA did not exhibit gelatinolytic activity. SDS/PAGE analysis indicated that the 72-kDa proMMP-2 was still intact even after a 24 h incubation. The proMMP-2-TIMP-2 complex inhibits active MMPs. Upon APMA treatment of the complex, the inhibitory activity of TIMP-2 against other MMPs was lost, suggesting that TIMP-2 interacts with the catalytic site of MMP-2 that is exposed upon APMA treatment. On the other hand, when the complex was treated with an equimolar amount of active MMP-3, proMMP-2 became activatable with APMA.

Incubation of proMMP-2 with MbAct converted the zymogen to a 67-kDa form, but the gelatinolytic activity was not observed. This was due to the inhibition of MMP-2 by TIMP-2 present in MbAct. By contrast, when proMMP-2 was incubated with MbAct in the presence of a substrate, gelatin, about 50% of MMP-2 activity was detected, suggesting that MbAct may participate in the activation of proMMP-2 even in the presence of TIMP-2. Incubation of the proMMP-2-TIMP-2 complex with MbAct also resulted in partial conversion of the zymogen to a 67-kDa form, but proteolytic activity was not observed. On the other hand, when the complex was reacted with an equimolar amount of MMP-3, MbAct generated about 25% of MMP-2 activity. These results indicate that blocking the TIMP-2 with an active MMP is essential for the activation of proMMP-2 when it is complexed with TIMP-2. Thus, even though the production of proMMP-2 is not altered by the connective tissue cells, the increased levels of active MMP-1, MMP-3, and other MMPs in the tissue may readily influence the amount of proMMP-2 available to be activated by MbAct. (supported by NIH Grant AR 39189 and AR 40994)