Tumor necrosis factor α (TNFα) induces pro-matrix metalloproteinase 9 production in human uterine cervical fibroblasts but interleukin 1α antagonizes the inductive effect of TNFα

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Abstract We have examined the regulation of precursor of matrix metalloproteinase 9 (proMMP-9)/progelatinase B production by tumor necrosis factor α (TNFα) and interleukin 1α (IL-1α) using human uterine cervical fibroblasts. TNFα, but not IL-1α, induces the production of proMMP-9 in the cervical cells. IL-1α, however, suppresses the TNFα-induced proMMP-9 production. 12-*O*-tetradecanoylphorbol 13-acetate (TPA) also stimulates the cervical cells to produce proMMP-9, and IL-1α synergistically enhances its production. TNFα-induced proMMP-9 production is not mediated by protein kinase C (PKC), whereas the effect of IL-1α is through PKC. By contrast, proMMP-3/prostromelysin 1 is up-regulated by TNFα or TPA in the presence of IL-1α, whose modulation is PKC-dependent. The suppressive effect of IL-1α on the TNFα-induced proMMP-9 production is a new biological effect of IL-1 on MMP production.

Key words: Matrix metalloproteinase 9; Progelatinase B; Tumor necrosis factor α; Interleukin 1α; Protein kinase C; Human uterine cervical fibroblast

1. Introduction

Matrix metalloproteinase 9 (MMP-9)/gelatinase B is a member of the MMP family. The enzyme has been isolated and characterized from a number of different cell types [1–3]. The expression of MMP-9 is implicated to both physiological and pathological breakdown of the connective tissue during invasion of cytotrophoblasts [4], the detachment of epithelial cells from basement membrane in the wound healing process [5], chronic inflammation such as rheumatoid arthritis [6,7] and tumor cell invasion and metastasis [8].

The synthesis of proMMP-9 has been shown to be regulated by 12-*O*-tetradecanoylphorbol 13-acetate (TPA), interleukin 1 (IL-1), tumor necrosis factor α (TNFα) and transforming growth factor β1 in various cell lines [1,5–8]. However, little evidence has been provided for the combined effect of those cytokines on the production of proMMP-9.

In the present study, we have examined the effects of TNFα and IL-1α on the synthesis of proMMP-9 using cultured human uterine cervical fibroblasts. Here we demonstrate that the production of proMMP-9 is markedly induced by TNFα, but not by IL-1α. On the other hand, the TNFα-induced proMMP-9 production is effectively suppressed by IL-1α.

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Abbreviations: MMP, matrix metalloproteinase; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; IL-1, interleukin 1; TNFα, tumor necrosis factor α; MEM, minimum essential medium; PKC, protein kinase C; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride; HA1004, *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride

2. Materials and methods

2.1. Cytokines and reagents

Recombinant human TNFα (rhTNFα; 2.55×10^6 U/mg) and recombinant human IL-1α (rhIL-1α; 2×10^7 U/mg) were kindly supplied by Dainippon Pharmaceutical Co., Suita, Osaka, Japan. MEM was purchased from Life Technologies, Inc., Grand Island, NY. Fetal bovine serum was from Whittaker Bioproducts Inc., Walkersville, MD. TPA, alkaline phosphatase-conjugated donkey anti-(sheep IgG)IgG, 5-bromo-4-chloro-3-indolyl phosphate, Nitro Blue Tetrazolium and staurosporine were from Sigma Chemical Company, St. Louis, MO. 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) and *N*-(2-guanidinoethyl)-5-isoquinolinesulfonamide hydrochloride (HA1004) were from Seikagaku Kogyo Co., Tokyo. All other chemicals were analytical reagents.

2.2. Cell culture and treatment

Human uterine cervical cells were prepared as described previously [9] and maintained in MEM/10% (v/v) fetal bovine serum until confluence. Confluent cervical fibroblasts were treated with cytokines and/or chemical compounds in serum-free MEM containing 0.2% (w/v) lactalbumin hydrolysate to examine the production of proMMPs. In this series of experiments, human uterine cervical fibroblasts were used at passage level from the 2nd to the 10th and the passage levels did not affect responsiveness to cytokines and chemical reagents. In addition, more than three experiments were repeated using cells at different passage levels with different sources.

2.3. Gelatin zymography

The activity of gelatinolytic enzyme in culture media was detected by electrophoresis in 7.5% (w/v) acrylamide gel co-polymerized gelatin (DIFCO Laboratories, Detroit, MI) at a final concentration of 0.6 mg/ml. Briefly, a 10 μl portion of harvested culture media was mixed with SDS-PAGE sample buffer [10] and then electrophoresed without boiling under non-reducing conditions. After electrophoresis, the gels were washed at room temperature for 1 h in 2.5% (v/v) Triton X-100/50 mM Tris-HCl/5 mM $CaCl_2$/1 μM $ZnCl_2$ (pH 7.5) to remove SDS and incubated at 37°C in the same buffer without Triton X-100, and then stained in 0.1% (w/v) Coomassie Brilliant Blue R-250. Clear zones of gelatin lysis against blue background stain indicated the presence of gelatinolytic enzyme.

2.4. Western blot analysis

ProMMP-9 in culture media was analyzed by Western blot analysis using polyclonal sheep anti-(human proMMP-9)antibody which was raised against proMMP-9 purified from HT-1080 fibrosarcoma cells as described previously [11]. Similarly, proMMP-3 was detected with polyclonal sheep anti-(human MMP-3)antibody [12]. The samples collected from triplicate wells of each treatment were concentrated with final 3.3% (w/v) trichloroacetic acid, dissolved in SDS-PAGE sample buffer in the presence of 2-mercaptoethanol, and subjected to SDS-PAGE (7.5% for proMMP-9 and 10% for proMMP-3). The proteins separated by SDS-PAGE were electrotransferred to a nitrocellulose

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filter, and the filter was reacted with sheep anti-(human proMMP-9) or anti-(human MMP-3)antibody which was then complexed with alkaline phosphatase-conjugated donkey anti-(sheep IgG)IgG. Immunoreactive proMMP-9 and proMMP-3 were visualized indirectly using 5-bromo-4-chloro-3-indolyl phosphate and Nitro Blue Tetrazolium as described previously [9].

3. Results

3.1. Induction of proMMP-9 in human uterine cervical fibroblasts by TNFα

The expression of gelatinolytic activities by human uterine cervical fibroblasts was first examined by gelatin zymography (Fig. 1). While the 72-kDa progelatinase (proMMP-2/progelatinase A) was constitutively expressed, a new gelatinolytic activity around 92 kDa was detected when the cells were treated with rhTNFα. TPA (10 nM), which induces proMMP-9 in several cell types [1], also stimulated the cells to produce the gelatinolytic activity of the same molecular mass. The 92-kDa gelatinolytic activity was completely inhibited by 5 mM EDTA, but not by 1 mM diisopropylfluorophosphate (data not shown). These results together with immunological studies indicate that the induced enzyme is proMMP-9 (see Fig. 3).

3.2. Suppression of the TNFα-induced production of proMMP-9 by IL-1α

Next we examined the combined effect of TNFα and IL-1α on the production of proMMP-9 in human uterine cervical fibroblasts. Unexpectedly, the co-treatment of the cells with rhTNFα and rhIL-1α suppressed the rhTNFα-induced proMMP-9 production in a dose-dependent manner, while rhIL-1α alone did not induce proMMP-9 (Fig. 2). Western blot analysis showed that the biosynthesis of proMMP-9 was increased by rhTNFα, which was decreased by rhIL-1α (Fig. 3A). By contrast, the production of proMMP-3 was further enhanced when the cells were treated with both rhTNFα and rhIL-1α (Fig. 3B).

On the other hand, the TPA-induced proMMP-9 was differently modulated by IL-1α. As shown in Figs. 2 and 3A, rhIL-1α synergistically enhanced the TPA-induced proMMP-9 production in a dose-dependent manner. Similarly, the TPA-induced production of proMMP-3 was augmented by rhIL-1α in a similar manner as in the case of the rhTNFα-treated cells. These results suggested that the regulation of proMMP-9 production by TNFα is different from that by TPA in human cervical fibroblasts, and that the suppressive effect of IL-1α is specific on the TNFα-induced production of proMMP-9.

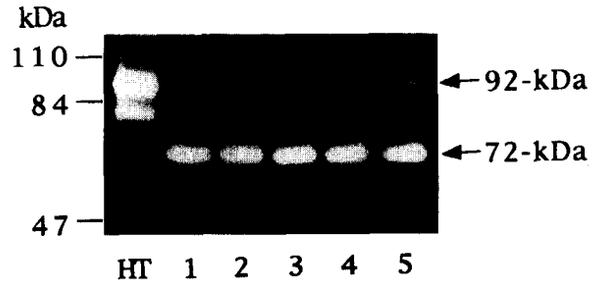

Fig. 1. TNFα induced the production of proMMP-9 in human uterine cervical fibroblasts. Confluent human uterine cervical fibroblasts at the 8th passage were treated with 0.2% (w/v) lactalbumin hydrolysate/MEM containing rhTNFα (0.001–10 ng/ml) and TPA (10 nM) for 2 days. Aliquots (10 μl) of harvested culture media were subjected to gelatin zymography as described in Section 2. More than three independent experiments were reproducible and typical data are shown. HT, pure proMMP-9 (1 ng/lane) of HT-1080 fibrosarcoma cells. Lane 1, control; lanes 2–4, cells treated with rhTNFα (0.001, 0.1 and 10 ng/ml, respectively); and lane 5, cells treated with TPA (10 nM).

Fig. 2. Regulation of the TNFα- and TPA-induced proMMP-9 production by IL-1α in human uterine cervical fibroblasts. Confluent cervical fibroblasts at the 6th passage were treated with rhTNFα (10 ng/ml), TPA (10 nM) and/or rhIL-1α (0.001-10 ng/ml) for 2 days, and the culture media were analyzed for the expression of gelatinolytic enzyme. Aliquots of the media (10 μl) were subjected to gelatin zymography to detect gelatinolytic activity as described in Section 2. More than three independent experiments were reproducible and typical data are shown. Lane 1, control; lane 2, rhTNFα (10 ng/ml); lanes 3–5, rhTNFα (10 ng/ml) plus rhIL-1α (0.001, 0.1 and 10 ng/ml, respectively); lane 6, TPA (10 nM); lanes 7–9, TPA (10 nM) plus rhIL-1α (0.001, 0.1 and 10 ng/ml, respectively); and lanes 10–12, rhIL-1α (0.001, 0.1 and 10 ng/ml, respectively).

3.3. Effect of staurosporine on the TNFα- and TPA-induced production of proMMPs-9 and -3 in the presence or the absence of IL-1α

Since TPA, a well characterized activator of protein kinase C (PKC) [13], is an effective enhancer of proMMP-9 production in our culture system (Fig. 1) and other cell systems [1], we examined whether activation of PKC is required for the expression of proMMP-9 in human uterine cervical fibroblasts. This was approached by using PKC inhibitor as shown in Table 1; staurosporine at concentrations of 5–20 nM did not affect the induction of proMMP-9 by rhTNFα, but the same concentrations of this compound caused a significant suppression of the TPA-induced proMMP-9 production with

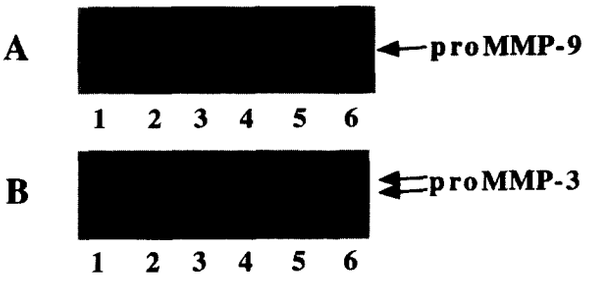

Fig. 3. Western blot analysis of proMMP-9 and proMMP-3 produced by human uterine cervical fibroblasts. Confluent cervical fibroblasts at the 3rd passage were treated with rhTNFα (10 ng/ml), TPA (10 nM) and/or rhIL-1α (10 ng/ml) for 18 h. The harvested culture media were subjected to Western blotting to detect proMMP-9 (A) and proMMP-3 (B) using the specific antibodies as described in Section 2. More than three independent experiments were reproducible and typical data are shown. The shadow visualized close to the main band corresponding to proMMP-9 was confirmed to be non-specific because it was also detected by non-immune sheep IgG instead of the antibody against proMMP-9. Lane 1, control; lane 2, rhTNFα (10 ng/ml); lane 3, rhTNFα (10 ng/ml) plus rhIL-1α (10 ng/ml); lane 4, TPA (10 nM); lane 5, TPA (10 nM) plus rhIL-1α (10 ng/ml); and lane 6, rhIL-1α (10 ng/ml).

Table 1
Effect of staurosporine on TNFα- and TPA-induced proMMPs-9 and -3 production in human uterine cervical fibroblasts

	Relative Induction (-fold)	
	proMMP-9	proMMP-3
Control	N.D.	1.0
rhTNFα (10 ng/ml)	1.0	7.6
+ Staurosporine (5 nM)	1.3[a]	5.1
+ Staurosporine (10 nM)	1.3[a]	2.7
+ Staurosporine (20 nM)	1.0[a]	2.1
TPA (10 nM)	1.0	3.1
+ Staurosporine (20 nM)	0.1[b]	1.3
Staurosporine (20 nM)	N.D.	0.8

Confluent cervical fibroblasts at the 6th passage were treated with rhTNFα (10 ng/ml) or TPA (10 nM) in the presence of staurosporine (5–20 nM) for 18 h. The harvested culture media were analyzed for the production of proMMP-9 and proMMP-3 by Western blot analysis. Their relative amounts were quantified by densitometric scanning and expressed taking the rhTNFα- or TPA-induced value as 1 for proMMP-9 and the control value of proMMP-3 as 1. Three independent experiments were reproducible and typical data are shown.
[a,b]Relative comparison against the rhTNFα- and TPA-induced proMMP-9, respectively. N.D., not detected.

a maximal inhibition of 86%. In contrast, rhTNFα-induced proMMP-3 production was inhibited by staurosporine with maximal inhibition of 83% (calculated after subtraction of the control value) at 20 nM. The TPA-induced proMMP-3 production was also decreased by 85% at the same concentration.

As shown in Table 2, staurosporine completely diminished the rhIL-1α-mediated suppression of the proMMP-9 production to the level of the cells treated with rhTNFα alone. The production of proMMP-3 enhanced by the co-treatment with rhTNFα and rhIL-1α was also decreased by staurosporine by 78% at the concentration of 20 nM. In addition, the enhanced production of proMMP-9 by the co-treatment of rhIL-1α and TPA was inhibited by staurosporine. Similar results were also obtained with another PKC inhibitor, H-7 but not with HA1004, a much weaker PKC inhibitor [14,15] (data not shown).

4. Discussion

IL-1α and TNFα are cytokines that evoke matrix degradation by inducing the responsive proteinases [16]. Our previous study [12] has shown that IL-1α stimulates the cervical cells to produce proMMPs-1 and -3. In this report, we demonstrated that the action of IL-1α on proMMP-9 production was different from that of TNFα: IL-1α preferentially modulates the production of proMMPs-1 and -3, but not that of proMMP-9; TNFα, on the other hand, functions as an effective inducer of the three proMMPs. In addition, while the production of proMMP-2 was not altered by treating the cells with TNFα, IL-1α and TPA, these treatments slightly stimulated the activation of proMMP-2 (Figs. 1 and 2). Recently, it has been shown that proMMP-2 is activated by a membrane associated MMP, MT-MMP [17]. Therefore, it is likely that the increase in the active form of MMP-2 after TNFα, IL-1α or TPA treatment may be mediated by an increased activity of MT-MMP. However, the combination of TNFα and IL-1α, and TPA and IL-1α did not change either the production or the activation of proMMP-2. Taken together these results indicate that the productions of proMMP-2, proMMP-3 and proMMP-9 by TNFα, IL-1α and TPA are differently regulated.

Little information is available regarding the signal transduction mechanism through which the production of proMMP-9 is mediated on cytokine stimulation. Recent studies have indicated that expression of proMMP-9 is regulated by PKC pathway in tumor cells [18,19] and T-lymphocytes [20,21]. In cervical fibroblasts, TNFα induced the production of proMMP-3 through PKC pathway as previous paper by Brenner et al. [22]. However, the TNFα-induced proMMP-9 production is independent of PKC activation. This was further confirmed by down-regulation of PKC in which the cells were pretreated with TPA (100 nM) for 24 h prior to stimulation with TNFα (T. Sato, A. Ito and Y. Mori, unpublished data). The PKC-independent signal pathway of TNFα has been reported for other cells [23–25]. Thus, we conclude that in cervical fibroblasts TNFα possesses at least two distinct signal transduction pathways after binding to its receptor: one is a PKC-independent pathway which leads to augmentation of the proMMP-9 production; and the other is the

Table 2
Involvement of PKC in IL-1α regulation of proMMPs-9 and -3 production in human uterine cervical fibroblasts

	Relative Induction (-fold)	
	proMMP-9	proMMP-3
Control	N.D.	1.0
rhTNFα (10 ng/ml)	1.0	10.2
rhTNFα (10 ng/ml) + rhIL-1α (10 ng/ml)	N.D.	16.6
+ Staurosporine (5 nM)	0.1[a]	14.5
+ Staurosporine (10 nM)	0.6[a]	7.1
+ Staurosporine (20 nM)	1.3[a]	4.5
TPA (10 nM)	1.0	3.4
TPA (10 nM) + rhIL-1α (10 ng/ml)	6.7[b]	13.1
+ Staurosporine (20 nM)	2.3[b]	4.1
rhIL-1α (10 ng/ml)	N.D.	8.3
Staurosporine (20 nM)	N.D.	0.9

Confluent cervical fibroblasts at the 3rd passage were treated with rhTNFα (10 ng/ml), TPA (10 nM) and/or rhIL-1α (10 ng/ml) in the presence of staurosporine (5–20 nM) for 18 h. The harvested culture media were analyzed for the production of proMMP-9 and proMMP-3 by Western blot analysis. Their relative amounts were quantified by densitometric scanning and expressed taking the rhTNFα- or TPA-induced value as 1 for proMMP-9 and the control value of proMMP-3 as 1. Three independent experiments were reproducible and typical data are shown.
[a,b]The relative comparison against the rhTNFα- and TPA-induced proMMP-9, respectively. N.D., not detected.

PKC-dependent pathway that stimulates the proMMP-1 and proMMP-3 gene expression.

A most striking finding in the present study is that the TNFα-induced proMMP-9 in the cervical fibroblasts was suppressed by IL-1α. Cytokines are known to cause various biological activities cooperatively rather than singly. In this regard, the TNFα-induced proMMP-3 in the cervical cells is further augmented in the presence of IL-1α. A similar observation was made on the proMMP-3 expression for rheumatoid human synovial fibroblasts [26]. However, this is not the case with proMMP-9. Although the suppressive mechanism of the TNFα-induced proMMP-9 by IL-1α cannot be explained at the moment, the antagonizing effect of IL-1α against the stimulatory activity of TNFα on proMMP-9 production shown here is a novel biological property of IL-1α.

In contrast to TNFα, TPA together with IL-1α synergistically enhanced the production of proMMP-9. A similar augmentation effect of TPA and IL-1 on proMMP-9 production was also observed in rabbit articular chondrocytes [11]. The difference between the effects of TNFα and TPA can be explained by distinct intracellular signal pathways, i.e., PKC dependency as demonstrated here (Table 1).

It has been reported that IL-1 induces the proMMP-9 production in human fibroblasts and in rabbit articular chondrocytes [6,27]. However, a significant induction of proMMP-9 by IL-1 and/or TNFα was not observed in human skin and synovial fibroblasts, although both cytokines enhanced the production of proMMPs-1 and -3 in those cells (T. Sato, A. Ito and Y. Mori, unpublished data). PKC activation was found to be necessary for the IL-1α-mediated induction of proMMP-3 and suppression of proMMP-9 (Table 2). The involvement of PKC in the IL-1α-regulated proMMP-3 production is consistent with our previous work [28]. Nevertheless, while IL-1α activates PKC and the stimulation of proMMP-9 production by combination of TPA and IL-1α is mediated by PKC, IL-1α alone does not evoke the proMMP-9 production in the cervical fibroblasts. The different cellular ability to produce a different set of proMMPs in response to cytokines is most likely reflected from cell specific intracellular signaling pathways.

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