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Urokinase Induces Matrix Metalloproteinase-9/ Gelatinase B Expression in THP-1 Monocytes via ERK1/2 and Cytosolic Phospholipase A_2 Activation and Eicosanoid Production

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Key Words
Eicosanoids · Matrix metalloproteinase · Migration · Phospholipase A_2 · Urokinase

Abstract
Objective: Urokinase-type plasminogen activator (uPA) regulates cell migration and invasion by pericellular proteolysis and signal transduction events. We characterized the mechanisms by which uPA regulates matrix metalloproteinase-9 (MMP9) function in THP-1 monocytes. ***Methods and Results:*** In THP-1 monocytes, MMP9 production induced by urokinase was completely inhibited by the ERK1/2 inhibitor, PD98059, but not by the p38 mitogen-activated protein kinase inhibitor, SB202190. A dominant negative MEK1 adenovirus also blocked MMP9 expression. The effect of urokinase was completely suppressed by genistein and by herbimycin A indicating that tyrosine kinase(s) are required for MMP9 production. Bisindolylmaleimide, a protein kinase C (PKC) inhibitor, did not decrease MMP9 expression suggesting that PKC activation is not required. Key roles for cytosolic phospholipase A_2 (PLA2) and eicosanoid production were shown by complete inhibition with methyl arachidonyl fluorophosphonate (an inhibitor of cytosolic PLA2), and indomethacin (a cyclooxygenase inhibitor), with no effect of monoalide, a secretory PLA2 inhibitor. uPA stimulated phosphorylation of cytosolic PLA2. ***Conclusions:*** Induction of MMP9 by uPA in THP-1 monocytes is via a pathway involving MEK1-ERK1/2-mediated activation of cytosolic PLA2 and eicosanoid generation. These data suggest important roles for eicosanoids in monocyte migration induced by uPA and MMP9.

Introduction

Matrix metalloproteinases (MMPs), a family of Zn-dependent neutral proteolytic enzymes, are known to participate in extracellular matrix remodeling and proteolytic processing of some growth factors and cytokines suggesting their role in many physiological and pathological processes [1]. The production and secretion of MMPs by different cell types regulated by growth factors, cytokines, and components of extracellular matrix are an obligatory component of normal and pathological processes related to cell migration and invasion [2].

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Among the MMP family, the type IV collagenases/gelatinases (MMP2 and MMP9) have some particularities in their physiological role because of their ability to disrupt type IV collagen, the predominant component of basement membrane [3]. A 72-kDa MMP2/gelatinase A is continuously expressed in some cell types [1]. A 92-kDa MMP9/gelatinase B is the inducible enzyme, which can be expressed in a number of cells under the action of TNF-α, IL-1β, PDGF and some other growth factors [4]. This expression has been shown to be mediated via ERK1/2, p38 mitogen-activated protein kinase (MAPK), and/or JNK signal transduction pathways [5]. Moreover, the involvement of cytosolic PLA2 (cPLA2) as well as cyclooxygenase reaction products, prostaglandins, has been demonstrated in several studies [6, 7].

Urokinase, in addition to its participating in fibrinolysis through plasminogen activation, is known to induce cell proliferation, migration and invasion [8], which could be mediated by the coupling of urokinase with its membrane receptor (uPAR) as well as by its proteolytic activity [9]. Stimulation of cell motility by urokinase and its receptor is mediated via signal transduction pathway(s) where tyrosine kinases, protein kinase C (PKC) and MAPK are involved [10, 11]. Therefore, a possibility exists for urokinase to induce the production of the enzymes participating in cellular activities potentiated by urokinase.

One of the possible targets of the regulatory action of urokinase could be MMP expression and activation. The urokinase-activated enzyme, plasmin, is known to activate proteolytically some members of the MMP family including MMP9. This activation is mediated via transforming of stromelysin into active enzyme, which, in turn, activates MMP9 [12]. In addition, MMP9 and urokinase are known to be coexpressed in some physiological and pathological states [13], suggesting a possibility for urokinase to induce production and secretion of this enzyme in some types of cells via signal transduction pathways.

We have recently shown that urokinase induces MMP9 secretion by THP-1 and U937 monocyte cells, the effect that does not depend on plasminogen activation and is due to MMP9 mRNA formation and protein synthesis [14, 15]. In the present study, we characterize the signal transduction mechanisms leading to MMP9 expression in THP-1 cells. Using the inhibitory analysis, we demonstrate the participation of the ERK1/2 MAPK cascade, tyrosine protein kinase(s), as well as cPLA2 and cyclooxygenase in MMP9 production induced by urokinase in THP-1 cells.

Methods

Recombinant uPA with wild-type structure (r-uPA^{wt}), proteolytically inactive urokinase with replacement of His^{204} in active center for Gln (r-uPA$^{H/Q}$) and proteolytically inactive urokinase with deletion of NH2-terminal 1–43 amino acids called 'growth-factor-like domain' (r-uPA$^{H/Q}$-GFD) were expressed in *Escherichia coli* and purified as described previously [16, 17]. Two-chain low-molecular-weight (LMW) urokinase with the catalytic domain (r-uPA^{LMW}) corresponding to 136–411 amino acids of r-uPA^{wt} was prepared by proteolytic cleavage of r-uPA^{wt} by plasmin, followed by purification with affinity chromatography, using monoclonal antibody against the urokinase catalytic domain coupled to CNBr-Sepharose 4B [18]. Recombinant kringle domain (rKD) corresponded to urokinase amino acids Glu^{43}-Arg^{156} and r-uPA-KD with deletions of these amino acids being expressed in *E. coli* and purified as described [18]. Methyl arachidonyl fluorophosphonate (MAFP) and monoalide were purchased from Biomol, Hamburg, Germany. Indomethacin and genistein were from Sigma (St. Louis, Mo., USA). PD 98059, SB 202190, SP600125 and bisindolylmaleimide (GF109203X) were from Calbiochem (San Diego, Calif., USA).

Cell Culture and Stimulation

THP-1 monocytes were cultured in RPMI 1640 medium containing penicillin/streptomycin with 10% fetal calf serum. Prior to experiments, the concentration of cells was adjusted to 5×10^5 cells/ml by suspension in RPMI 1640 containing 0.1% FCS. In each experiment, the cells were exposed to uPA (20 nM) or saline for 48 h. Inhibitors were added to the cells 1 h before urokinase. Following incubation, conditioned media were collected and immediately frozen until zymography for gelatinase activity was performed. The control cells as well as cells treated with uPA were used for total RNA isolation and RT-PCR cDNA amplification.

Recombinant Adenoviruses and Cell Infection

Full-length dominant-negative (DN) MEK1 and β-galactosidase (LacZ) gene were cloned into a pAd-CMV-V5-Dest vector (Invitrogen, Carlsbad, Calif., USA). The recombinant adenoviruses were expanded in HEK 293 cells and purified by cesium chloride ultracentrifugation. The purified viruses were stored in 10% (v/v) glycerol/phosphate-buffered saline at –80°C. THP-1 cells were infected at 300, 100 and 30 multiplicity of infection followed by 72-hour incubation at 37°C in a CO_2 incubator. Thereafter, the cells were suspended in 0.1%FCS/RPMI 1640 and stimulated by uPA. β-Galactosidase activity measured in LacZ-transduced THP-1 cell lysates using standard kits (Promega, Madison, Wisc., USA) was proportional to multiplicity of cell infection by adenovirus.

Immunoblot Analysis of MAPK (ERK) Phosphorylation

Phosphorylation of ERK1/2 was determined by Western blotting (New England Biolabs, Beverly, Mass., USA). THP-1 cells were treated with uPA in RPMI 1640 with 0.1% FBS for 5, 10 and 30 min. Cells were lysed in 100 μl of sample buffer (0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol (v/v), 5% β-mercaptoethanol, 0.001% bromophenol blue), sonicated, electrophoresed in 10% SDS-polyacrylamide gel, and transferred to nitrocellulose membranes. The blots were probed with primary anti-phospho-ERK1/2 antibody for the first membrane and anti-ERK1/2 for the second

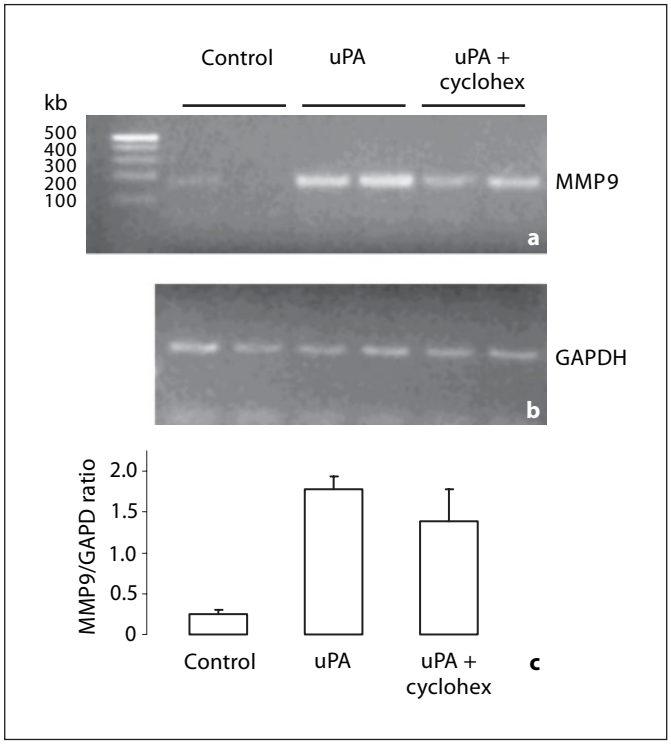

Fig. 1. Urokinase induces MMP9 mRNA formation in THP-1 cells. THP-1 cells were incubated for 48 h either with saline (control), 20 nM r-uPA, or 0.5 μg/ml cycloheximide and 20 nM r-uPA (uPA+cyclohex) as indicated, following by RNA isolation and RT-PCR analysis of human MMP9 and GAPDH mRNAs. **a**, **b** MMP9 (**a**) and GAPDH (**b**) PCR products were obtained with corresponding gene-specific primer pairs during 35-cycle amplification of cDNA synthesized on 30 ng of THP-1 total RNA. **c** MMP9/GAPD ratios obtained by scanning densitometry of RT-PCR data.

membrane, and secondary antibody in dilutions recommended by the manufacturer. ERK1/2 activation was measured as a ratio of the intensities of phospho-ERK1/2 bands to those of total ERK1/2, and expressed relative to that in unstimulated cells.

Immunoblot Analysis of cPLA2 Phosphorylation

Phosphorylation of cPLA2 was determined by Western blotting using anti-phospho-cPLA2 and anti-cPLA2 antibodies (Cell Signaling Technology). THP-1 monocytes were incubated for 24 h in RPMI 1640 containing 0.1% FBS. In experiments, THP-1 cells were treated with uPA (20 nM) in RPMI 1640 without FBS for 10 and 30 min, and 1, 4 and 14 h. Western blotting was performed with PVDF membranes. cPLA2 activation was measured as a ratio of the intensities of phospho-cPLA2 bands to those of total cPLA2.

Gelatin Zymography

Cell-conditioned media were mixed with sample buffer without β-mercaptoethanol and loaded on 7.5% SDS-polyacrylamide gel containing 0.1% gelatin. After electrophoresis, the gels were washed 3 times with 100 ml of 2.5% Triton X-100 and incubated for 18 h in a buffer consisting of 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.05% Brij 35, and 10 mM $CaCl_2$. After incubation, the gels were stained with 0.25% Coomassie brilliant blue G-250 in 40% (v/v) methanol and 10% (v/v) acetic acid, followed by washing off the excessive dye by a mixture of 40% (v/v) methanol and 10% (v/v) acetic acid. Proteolytic activity was detected by evaluating the expression of unstained bands on the gelatin-stained background. Zymograms were subjected to densitometric analysis using PCBAS software. Gelatinolytic activity was expressed in arbitrary units.

RNA Extraction and RT-PCR

Total RNA was isolated from THP-1 cells using Qiagen RNAeasy miniprep columns. The integrity of the samples was verified by 1.5% agarose gel electrophoresis. Aliquots were stored in RNase-free sterile tubes until use. Using oligonucleotide primer pairs for MMP9 (reverse 5′-GGAATGATCTAAGCCCAGCG-3′, forward 5′-GTGCGTCTTCCCCTTCACTTTCCT-3′) and for GAPDH (reverse 5′-TCCACCACCCTGTTGCTGTA-3′, forward 5′-ACCACAGTCCATGCCATCAC-3′) [15], 100 ng of total RNA was reverse transcribed and PCR amplified in accordance with the Perkin Elmer Gene Amp® protocol (Roche Molecular Systems, Branchburg, N.J., USA). The thermal profile involved 35 cycles of denaturation at 95°C for 60 s, primer annealing at 60°C for 60 s, and extension at 60°C for 60 s. Appropriate controls (no reverse transcriptase and no template controls) were included. 6 μl of each PCR mixture was run on 1.5% agarose gel in Tris-borate-EDTA electrophoresis buffer with ethidium bromide (1 μg/ml). The fluorescence-stained bands corresponding to the PCR products were quantified using the AlphaImager™ System (Alpha Innotech).

Results

MMP9 mRNA Formation in THP-1 Cells Induced by Urokinase Is Insensitive to Protein Synthesis Inhibition

Urokinase stimulates THP-1 and U937 monocyte cells to produce and release a 92-kDa MMP, MMP9/gelatinase B in a time- and concentration-dependent manner as measured by zymography and immunoblotting [14, 15]. To define the mechanisms for MMP9 induction, we analyzed the expression of MMP mRNA by RT-PCR. As shown in figure 1, MMP9 mRNA expression was induced by uPA, and was poorly affected by cycloheximide, suggesting that the synthesis of intermediary protein factor(s) is not obligatory for MMP9 promoter activation in THP-1 cells.

Participation of the Structural Domains of uPA in the Induction of MMP9 Production by THP-1 Monocytes

We have shown previously that MMP9 expression in THP-1 cells treated with urokinase is not mediated by

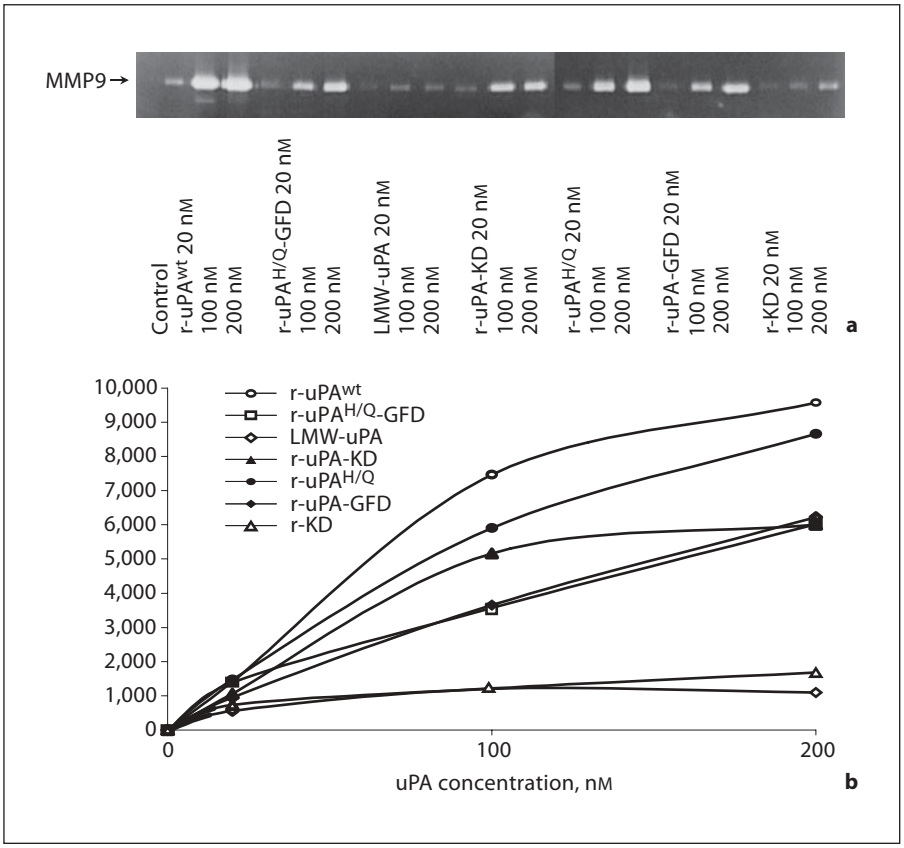

Fig. 2. The dependence of MMP9 production by THP-1 cells on urokinase functional domains. THP-1 cells were incubated with recombinant uPA forms for 48 h followed by zymography of MMP9 in conditioned media. The following uPA variants were used: r-uPA^{wt} (wild-type uPA); r-uPA$^{H/Q}$ (substitution of His^{204}→Gln^{204} in active center); r-uPA-GFD (lacking GFD); r-uPA$^{H/Q}$-GFD (absence of GFD and catalytic activity); r-uPA-KD (lacking KD); LMW-uPA (LMW uPA containing no GFD and KD), and r-KD (containing only KD). **a** Typical zymograms obtained after THP-1 cell incubation with uPA recombinants. **b** Concentration dependence of MMP9 production obtained by scanning densitometry of zymograms. Data are representative of three independent experiments.

plasmin formation and, moreover, is not reproduced by tissue-type plasminogen activator. In addition, catalytic activity of urokinase is not required for MMP9 production [14, 15]. To define the role of other uPA structural domains in induction of MMP9 expression in monocytes, we measured MMP9 release by THP-1 cells stimulated with the recombinant forms of uPA: 'wild-type' uPA (r-uPA, r-uPA^{wt}) with unmodified primary structure; r-uPA$^{H/Q}$ having no catalytic activity due to replacement of His^{204} to Gln^{204} in the active center; r-uPA-GFD lacking GFD; r-uPA$^{H/Q}$-GFD with no GFD and catalytic activity; r-uPA-KD lacking the KD; LMW-uPA having an unmodified catalytic site but containing no GFD and KD, and r-KD containing only KD. Among the uPA constructs tested, the 'wild-type' uPA had the highest ability to induce MMP9 release by THP-1 monocytes (fig. 2). r-uPA$^{H/Q}$, r-uPA-GFD, r-uPA$^{H/Q}$-GFD and r-uPA-KD were also able to stimulate MMP9 release, but were weaker compared with r-uPA (40–80%) when 20 nM of the proteins were added to THP-1 cells. LMW-uPA and r-KD had no ability to induce MMP9 release by THP-1 cells. These data clearly indicate that in spite of catalytic activity, the possibility to contact the cell surface via the KD and/or the GFD is critical for MMP9 production induced by urokinase in THP-1 cells.

Urokinase Induces MMP9 Expression via MAPK and Tyrosine Kinase Pathways but Not via PKC Activation

The expression of MMPs in different cell types is known to be stimulated by growth factors and cytokines via mechanisms that can involve extracellular-signal-regulated kinase (ERK1/2), p38-mitogen-activated kinase and c-Jun N-terminal kinase (JNK), mechanisms that vary depending on the growth factor and cell type [5]. To explore which of these pathways affect urokinase-induced MMP9 expression in THP-1 monocytes, we first investigated the involvement of ERK1/2. Figure 3 demonstrates that r-uPA stimulated phosphorylation and activation of ERK1/2. A selective inhibitor of MEK-1, PD98059, completely prevented its activation by uPA. Similarly, PD98059 completely blocked the r-uPA-induced increase in MMP9 released (table 1). In contrast, the p38 MAPK inhibitor SB202190 (25 μM) as well as the JNK inhibitor SP600125 (10 μM) were without effect (table 1).

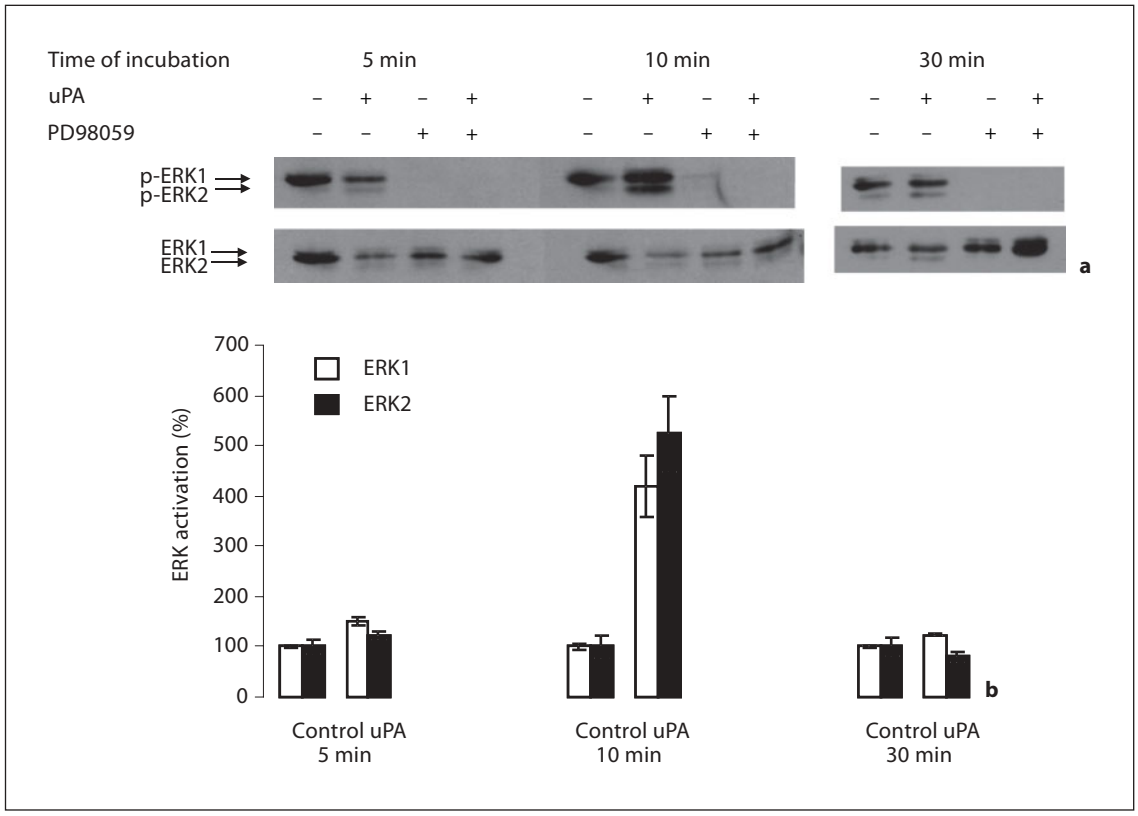

Fig. 3. Activation of ERK1/2 by urokinase. **a** Western blots of phosphorylated ERK1/2 (p-ERK1/2) and total ERK1/2 of lysates from THP-1 cells incubated with 25 μM PD98059 and 20 nM r-uPA. **b** Relative levels of ERK1 and ERK2 activation quantitated by scanning densitometry and normalized to the levels of total ERK1 and ERK2, respectively. Means ± SE of three independent experiments are represented.

The involvement of the MEK1-ERK1/2 pathway in MMP9 expression by urokinase was also tested in THP-1 cells infected with an adenoviral construct encoding DN-MEK1. The construct encoding β-galactosidase (LacZ) was used as a control. Figure 4 shows that the cells infected by virus (multiplicity of infection 100 and 300) have a dramatic decrease in uPA-induced MMP9 secretion (fig. 4a) and ERK1/2 activation (fig. 4b) compared to control adenoviral construct (DN-MEK1-300uPA vs. LacZ-300uPA; DN-MEK1-100uPA vs. LacZ-100uPA) indicating that suppressing ERK1/2 activation by MEK1 inhibits uPA-mediated MMP9 production (fig. 4c).

MMP9 secretion in THP-1 cells treated with uPA was also dramatically suppressed by the tyrosine kinase inhibitors genistein (10 μM, table 1) and herbimycin A (2 μM, data not shown) indicating that tyrosine kinase(s) participate in signal transduction pathways mediating MMP9 upregulation by urokinase. Inhibition of PKC by

Table 1. Effect of protein kinase, phospholipase and cyclooxygenase inhibitors on uPA-induced MMP9 production in THP-1 cells

Treatment	MMP9 activity arbitrary units	Inhibition %
uPA	1,095 ± 9	NA
PD98059 25 μM	21 ± 2	98 ± 0
SB202190 25 μM	1,075 ± 57	2 ± 5
SP600125 10 μM	1,021 ± 89	7 ± 8
Genistein 10 μM, uPA	7 ± 1	99 ± 0
BIM 2.5 μM	1,054 ± 131	4 ± 12
MAFP 2 μM	361 ± 20	67 ± 2
Monoalide 0.4 μM	1,238 ± 160	–13 ± 14
Indomethacin 30 μM	45 ± 5	96 ± 1

BIM = Bisindolylmaleimide; NA = not assessed.

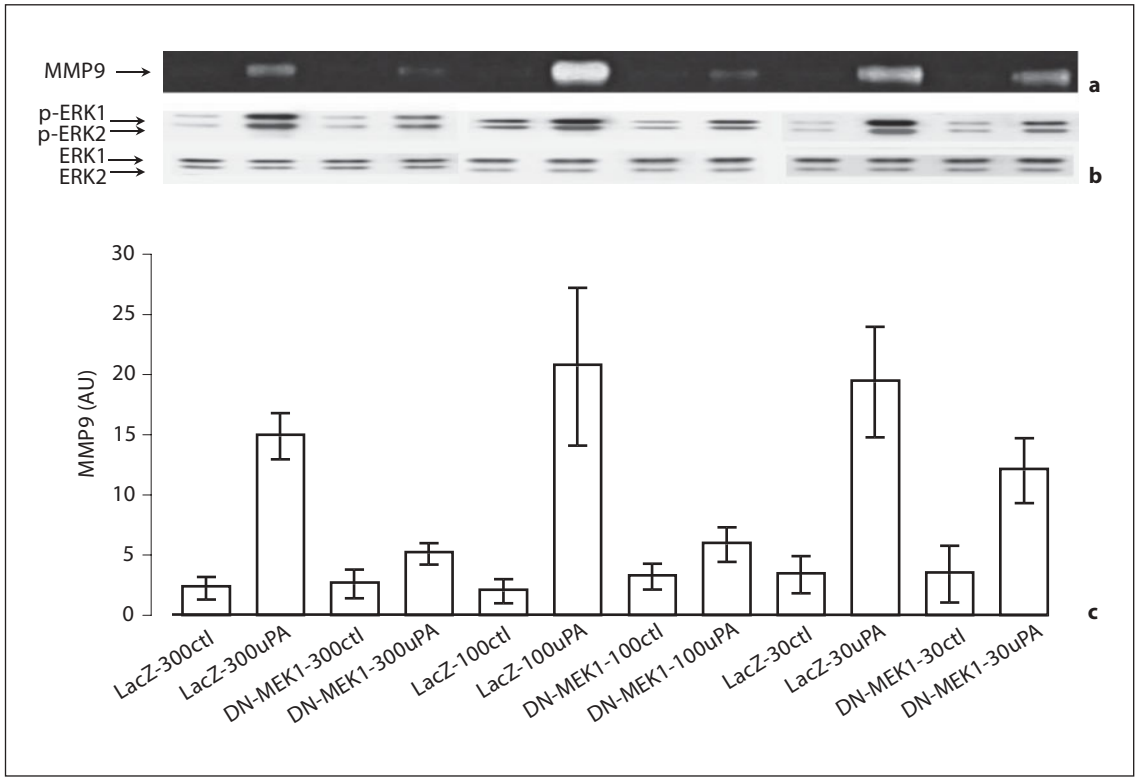

Fig. 4. The effect of THP-1 cell infection by adenoviral construct encoding DN-MEK1 or β-galactosidase (LacZ) on MMP9 production and ERK1/ERK2 activation induced by urokinase. The cells were infected with DN-MEK1 or LacZ encoding adenoviral construct at 300, 100, and 30 multiplicity of infection for 72 h. **a**, **c** Following infection, cells were incubated with saline (LacZ-ctl, DN-MEK1-ctl) or 20 nM uPA (LacZ-uPA, DN-MEK1-uPA) for 48 h in 0.1% FBS/RPMI 1640. MMP9 production was analyzed by zymography. Typical presentation of zymography data (**a**) and means ± SE of 4 experiments (**c**) are shown. **b** pERK1/2 and ERK1/2 were measured in lysates of THP-1 cells infected with DN-MEK1 or LacZ following 10-min stimulation by 20 nM urokinase. AU = Arbitrary units.

bisindolylmaleimide (2.5 μM) did not alter MMP9 secretion (table 1), suggesting that PKC activation by diacylglycerol, the product of phospholipase C activity, is not involved in the signal transduction pathway resulting in MMP9 expression induced by urokinase.

The Effect of Urokinase Is Mediated via PLA2 Activation and Eicosanoid Formation

To evaluate the role of arachidonate metabolites in MMP9 upregulation induced by urokinase, we tested the effects of specific inhibitors of PLA2, the enzyme liberating arachidonic acid from phospholipids. MAFP, which selectively inhibits cPLA2 (IC_{50} = 2 μM), inhibited MMP9 expression induced by urokinase by ~60%, at 2–4 μM (table 1). When used at 10 μM concentration and higher, MAFP induced the death of THP-1 cells revealed by light microscopy and trypan blue inclusion (data not shown). Monoalide, an inhibitor selective for secretory PLA2 (sPLA2; IC_{50} = 0.2 μM), had no effect when added at 0.4 μM (table 1). In addition, figure 5 demonstrates that r-uPA stimulated phosphorylation and activation of cytosolic PLA2. This activation reaches a maximum 1 h after incubating cells in the presence of urokinase. These data indicate that cPLA2 but not sPLA2 is required for the signal transduction pathway leading to MMP9 expression in THP-1 cells stimulated by urokinase.

The effect of uPA was dramatically inhibited by 30 μM indomethacin, a cyclooxygenase inhibitor (table 1). This finding indicates that the next step involved in this signaling cascade is formation of prostaglandins, the substances known to induce MMP9 expression [7].

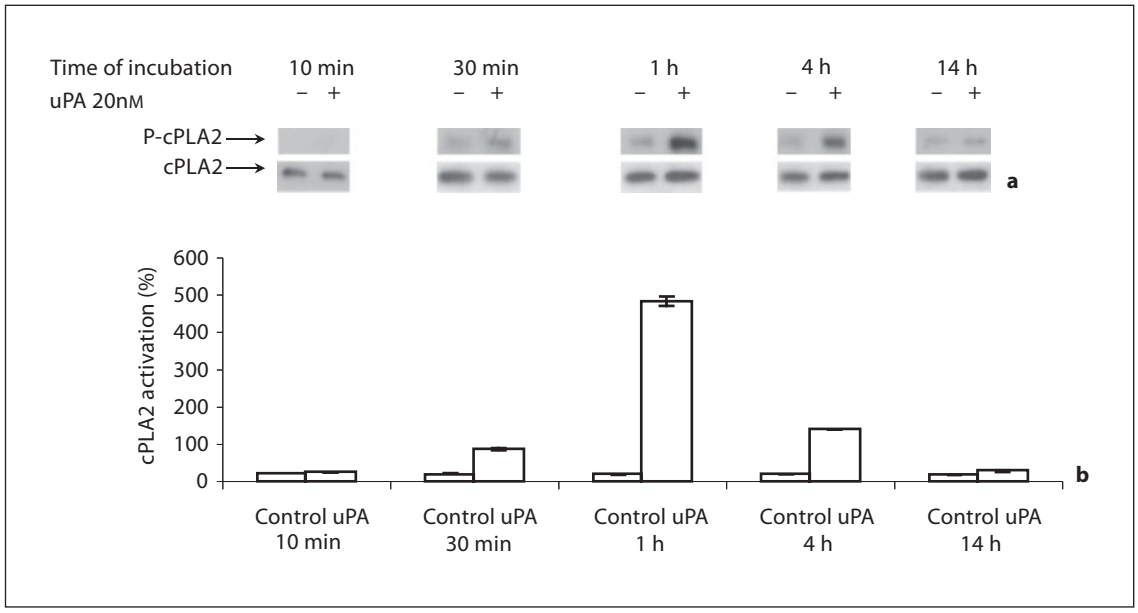

Fig. 5. Activation of cPLA2 by urokinase in THP-1 cells. **a** Western blots of phosphorylated cPLA2 (P-cPLA2) and total cPLA2 of lysates from THP-1 cells incubated with 20 nM r-uPA. **b** Relative levels of cPLA2 activation quantitated by scanning densitometry and normalized to levels of total cPLA2. Data are means ± SE of three independent experiments.

Discussion

Evidence is emerging that urokinase, a component of fibrinolytic system, is implicated in biological processes related to cell proliferation, motility and invasion. The ability of cells to penetrate through extracellular barriers during invasion is significantly dependent on pericellular proteolysis which is determined by the membrane location of the urokinase coupled with its membrane receptor, glycosyl-phosphatidylinositol-anchored protein [19]. In addition, plasmin, created by urokinase from plasminogen, is crucial for cell migration and invasion. Our studies indicated that urokinase, when incubated with THP-1 monocyte cells, induced gene transcription, protein synthesis and secretion of MMP9 [15]. This effect is not reproduced by tissue-type plasminogen activator, is not related to proteolytic activation of MMP9, and is not suppressed by plasmin inhibitors [14], suggesting that plasminogen activation is not implicated in MMP9 expression by THP-1 cells. In total, these data suggest that some other factors, besides the catalytic activity of urokinase, could be involved in MMP9 upregulation.

Indeed, the loss of the catalytic activity of uPA due to replacement of His^{204} in the active center to Gln preserves in part its ability to induce MMP9 expression in THP-1 monocytes. uPA variants lacking GFD (r-$uPA^{H/Q}$-GFD) or KD (r-uPA-GFD) are also able to induce MMP9 expression. At the same time, LMW urokinase (with missing GFD and KD), in spite of preserved catalytic activity, failed to induce the expression of MMP9.

The Scatchard plot data obtained in our laboratory [18] demonstrated that wild-type uPA with unmodified molecular structure specifically binds to two different classes of binding sites on the cell membrane having high and low affinity. The high-affinity interaction of urokinase with the cell membrane is known to reflect uPA coupling with its membrane receptor via GFD, while KD mediates uPA interaction with low-affinity sites of the cellular membrane [18]. Taken together, these data indicate that the ability of uPA to induce MMP9 expression in THP-1 cells is determined by its interaction with the cell membrane via GFD and/or KD. This interaction is more critical for MMP9 expression than the catalytic activity of urokinase.

The interaction of urokinase with the cell surface can result in signal transduction processes in which some intracellular proteins are involved. Urokinase, together with its membrane receptor, was shown to coprecipitate

with Src family kinases, nucleolin and casein kinase-2, ERK1/2 [20, 21], and some other components of intracellular signaling. This interaction might result in activation of signaling pathways via tyrosine phosphorylation of intracellular proteins, generating a high level of active ERK1/2 necessary for tumor growth in vivo [22] and some other intracellular signaling events.

Our study has shown that urokinase induces MMP9 expression via ERK1/2 activation and that p38 MAPK as well as JNK pathways are not involved in this stimulation. When stimulated by urokinase, THP-1 monocytes secrete MMP9 via ERK1/2 cascade activation. The absence of the effect of bisindolylmaleimide, the PKC inhibitor, indicates that PKC activation is not implicated in this process. There are several studies demonstrating MMP9 expression via p38 MAPK, ERK1/2 and JNK activation by cytokines and growth factors in different cell types [5]. MMP9 upregulation can be mediated via mobilizing of AP-1 and NFκB transcriptional factors which have been characterized in the MMP9 promoter [23].

Several experimental findings indicate that the formation of arachidonic acid in the reaction catalyzed by PLA2 following eicosanoid synthesis is another step of intracellular signal transduction implicated in a wide number of cellular activities. In the present study, MMP9 expression in THP-1 monocytes was dramatically suppressed by inhibition of cPLA2, and is not sensitive to secretory PLA2 inhibition, suggesting that the effect of urokinase is mediated through the activation of cPLA2. In addition, participation of cPLA2 in signal transduction mechanisms of MMP expression was demonstrated in human monocytes stimulated by lipopolysaccharide [6]. At least two possibilities exist for cPLA2 mobilizing in cells; one of them is related to cPLA2 phosphorylation on several specific sites. cPLA2 can be phosphorylated on serine residues by either ERK1/2 or PKC resulting in an increase in catalytic cPLA2-specific activity. ERK1/2 selectively phosphorylates the domain of cPLA2 containing a MAPK consensus sequence, whereas PKC phosphorylates sites in all three recombinantly expressed domains of the enzyme. Peptide mapping indicates that the site phosphorylated by ERK1/2 is different from those phosphorylated by PKC [24]. In addition, cPLA2 can be phosphorylated by tyrosine kinase, and this type of phosphorylation is involved in the activation of this enzyme in Madin-Darby canine kidney cells induced by bradykinin [25]. MMP expression in human peripheral blood monocytes induced by lipopolysaccharide is mediated by tyrosine phosphorylation of cPLA2 [6]. In our study, the possibility for participation of PKC was excluded. However, activation of cPLA2 by ERK1/2 or by tyrosine kinase could be implicated in this activation.

Arachidonic acid, by itself or via metabolizing through cyclooxygenase and lipoxygenase pathways, regulates transcriptional activation of gene expression in cell activation, differentiation and tissue development. Our data indicate that MMP9 upregulation by urokinase is inhibited by indomethacin, suggesting the involvement of cyclooxygenase metabolites in this process. This finding is in accordance with the mediator role of prostaglandin E_2 in the stimulation of MMP expression by lipopolysaccharide in human monocytes [4, 6]. The direct upregulation of MMP9 mRNA transcription and protein synthesis by prostaglandin E_2 was demonstrated in human T cells [7].

In conclusion, our studies indicate that urokinase, in addition to its other known effects on tissue remodeling and repair, can also stimulate monocytes to produce latent MMP9 via mechanisms dependent on ERK1/2 activation and cytosolic PLA2 activity. These effects of urokinase are dependent on its interaction with cell membrane as well as on its catalytic activity. This mechanism could be important for MMP9 production in some pathological conditions where coexpression of urokinase and MMP9 is observed [13]. Further experiments should provide more information about the details of this signaling process and about its physiological and pathological significance.

Acknowledgments

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References

1 Newby AC: Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 2005;85:1–31.

2 Kennedy SH, Qin H, Lin L, Tan EML: Basic fibroblast growth factor regulates type I collagen and collagenase gene expression in human smooth muscle cells. Am J Pathol 1995; 146:764–771.

3 Kleinman HK, McGarvey ML, Hassel JR, Star VL, Cannon FB, Laurie GW, Martin GR: Basement membrane complexes with biological activity. Biochemistry 1986;25:312–318.

4 Zhang Y, McCluskey K, Fujii K, Wahl LM: Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-α, granulocyte-macrophage CSF, and IL-1β through prostaglandin-dependent and -independent mechanisms. J Immunol 1998;161:3071–3076.

5 Cho A, Graves J, Reidy M: Mitogen-activated protein kinases mediate matrix metalloproteinase-9 expression in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2000;20:2527–2532.

6 Shankavarah UT, DeWitt DL, Wahl LM: Lipopolysaccharide induction of monocyte matrix metalloproteinases is regulated by the tyrosine phosphorylation of cytosolic phospholipase A2. J Leukoc Biol 1998;64: 221–227.

7 Zeng L, An S, Goetzl J: Regulation of expression of matrix metalloproteinase-9 in early human T cells of the HSB.2 cultured line by the EP_3 subtype of prostaglandin E_2 receptor. J Biol Chem 1996;271:27744–27750.

8 Kanse SM, Benzakour O, Kanthou C, Kost C, Lijnen HR, Preissner KT: Induction of vascular SMC proliferation by urokinase indicates a novel mechanism of action in vasoproliferative disorders. Arterioscler Thromb Vasc Biol 1997;17:2848–2854.

9 Behrendt N, Ronne E, Dano K: The structure and function of the urokinase receptor, a membrane protein governing plasminogen activation on the cell surface. Biol Chem Hoppe Seyler 1995;376:269–279.

10 Busso N, Masur SK, Lazega D, Waxman S, Ossowski L: Induction of cell migration by pro-urokinase binding to its receptor: possible mechanism for signal transduction in human epithelial cells. J Cell Biol 1994;126: 259–270.

11 Tang H, Kerins DM, Hao Q, Inagami T, Vaughan DE: The urokinase-type plasminogen activator receptor mediates tyrosine phosphorylation of focal adhesion proteins and activation of mitogen-activated protein kinase in cultured endothelial cells. J Biol Chem 1998;273:18268–18272.

12 Ramos-DeSimone N, Hahn-Dantona E, Sipley J, Nagase H, French DL, Quigley JP: Activation of matrix metalloproteinase-9 (MMP-9) via converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. J Biol Chem 1999;274:13066–13076.

13 Inuzuka K, Ogata Y, Nagase H, Shirouzu K: Significance of coexpression of urokinase-type plasminogen activator, and matrix metalloproteinase 3 (stromelysin) and 9 (gelatinase B) in colorectal carcinoma. J Surg Res 2000;93:211–218.

14 Menshikov MYu, Elizarova EP, Kudryashova E, Timofeeva AV, Khaspekov GL, Beabealashvilli Rsh, Bobik A: Plasmin-independent gelatinase B (matrix metalloproteinase-9) release by monocytes under the influence of urokinase. Biochemistry (Moscow) 2001;66:954–959.

15 Menshikov M, Elizarova E, Plakida K, Timofeeva A, Khaspekov G, Beabealashvilli R, Bobik A, Tkachuk V: Urokinase upregulates matrix metalloproteinase-9 expression in THP-1 monocytes via gene transcription and protein synthesis. Biochem J 2002;367: 833–839.

16 Stepanova V, Bobik A, Bibilashvili R, Belogurov A, Rybalkin I, Domogatsky S, Little PJ, Goncharova E, Tkachuk V: Urokinase plasminogen activator induces smooth muscle cell migration: key role of growth factor-like domain. FEBS Lett 1997;414:471–474.

17 Poliakov AA, Mukhina SA, Traktouev DO, Bibilashvily RSh, Gursky YG, Minashkin MM, Stepanova VV, Tkachuk VA: Chemotactic effect of urokinase plasminogen activator: a major role for mechanisms independent of its proteolytic or growth factor domains. J Recept Signal Transduct Res 1999;19:939–951.

18 Mukhina S, Stepanova V, Traktouev D, Poliakov A, Beabealashvilly R, Gursky Y, Minashkin M, Shevelev A, Tkachuk V: The chemotactic action of urokinase on smooth muscle cells is dependent on its kringle domain. Characterization of interactions and contribution to chemotaxis. J Biol Chem 2000;275:16450–16458.

19 Gyetko MR, Todd RF 3rd, Wilkinson CC, Sitrin RG: The urokinase receptor is required for human monocyte chemotaxis in vitro. J Clin Invest 1994;93:1380–1387.

20 Rabbani SA, Mazar AP, Bernier SM, Haq M, Bolivar I, Henkin J, Goltzman D: Structural requirements for the growth factor activity of the amino-terminal domain of urokinase. J Biol Chem 1992;267:14151–14156.

21 Konakova M, Hucho F, Schleuning WD: Downstream targets of urokinase-type plasminogen-activator-mediated signal transduction. Eur J Biochem 1998;253:421–429.

22 Aguirre-Ghiso JA, Liu D, Mignatti A, Kovalski K, Ossowski L: Urokinase receptor and fibronectin regulate the ERK(MAPK) to p38(MAPK) activity ratios that determine carcinoma cell proliferation or dormancy in vivo. Mol Biol Cell 2001;12:863–879.

23 Gum R, Lengyel E, Juarez J, Chen JH, Sato H, Seiki M, Boyd D: Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase kinase-1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. J Biol Chem 1996;271:10672–10680.

24 Nemenoff RA, Winitz S, Qian NX, Van Putten V, Johnson GL, Heasley LE: Phosphorylation and activation of a high molecular weight form of phospholipase A2 by p42 microtubule-associated protein 2 kinase and protein kinase C. J Biol Chem 1993;268: 1960–1964.

25 Xing M, Tao L, Insel PA: Role of extracellular signal-regulated kinase and PKC alpha in cytosolic PLA2 activation by bradykinin in MDCK-D1 cells. Am J Physiol 1997;272: C1380–C1387.