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Induction of Stromelysin-1 (MMP-3) by Fibroblast Growth Factor-2 (FGF-2) in FGF-$2^{-/-}$ Microvascular Endothelial Cells Requires Prolonged Activation of Extracellular Signal-Regulated Kinases-1 and -2 (ERK-1/2)

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Abstract Basic fibroblast growth factor (FGF-2) and matrix metalloproteinases (MMPs) play key roles in vascular remodeling. Because FGF-2 controls a number of proteolytic activities in various cell types, we tested its effect on vascular endothelial cell expression of MMP-3 (stromelysin-1), a broad-spectrum proteinase implicated in coronary atherosclerosis. Endothelial cells (EC) from FGF-$2^{-/-}$ mice are highly responsive to exogenous FGF-2 and were therefore used for this study. The results showed that treatment of microvascular EC with human recombinant FGF-2 results in strong induction of MMP-3 mRNA and protein expression. Upregulation of MMP-3 mRNA by FGF-2 requires de novo protein synthesis and activation of the ERK-1/2 pathway. FGF-2 concentrations (5–10 ng/ml) that induce rapid and prolonged (24 h) activation of ERK-1/2 upregulate MMP-3 expression. In contrast, lower concentrations (1–2 ng/ml) that induce robust but transient (<8 h) ERK-1/2 activation are ineffective. Inhibition of ERK-1/2 activation at different times (−0.5 h to +8 h) of EC treatment with effective FGF-2 concentrations blocks MMP-3 upregulation. Thus, FGF-2 induces EC expression of MMP-3 with a threshold dose effect that requires sustained activation of the ERK-1/2 pathway. Because FGF-2 controls other EC functions with a linear dose effect, these features indicate a unique role of MMP-3 in vascular remodeling. J. Cell. Biochem. 90: 1015–1025, 2003.

Key words: endothelial cells; fibroblast growth factors; matrix-metalloproteinases; signal transduction

Vascular remodeling is characterized by cell proliferation and migration, and changes in the composition of the extracellular matrix (ECM). These effects are mediated by a variety of growth factors and ECM-degrading proteinases [Schwartz et al., 1995]. Among the growth factors involved in this process, basic fibroblast growth factor (FGF-2) plays a prominent role [Lindner and Reidy, 1991; Nguyen et al., 1994].

FGF-2, the prototypic member of a large family of heparin-binding polypeptides and a potent angiogenesis inducer, modulates vascular endothelial cell proliferation, migration, and proteinase production [Bikfalvi et al., 1997]. FGF-2 initiates intracellular signaling in a variety of cell types, including EC, through a dual receptor system consisting of receptor tyrosine kinases (FGFRs) and heparan sulfate proteoglycans [Moscatelli, 1987; Spivak-Kroizman et al., 1994; Bikfalvi et al., 1997]. FGF-2 binding to FGFRs results in activation of various intracellular signaling pathways including the

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extracellular signal regulated kinase (ERK) and phosphatidyl-inositol-3 kinase (PI-3K) pathways [Karin, 1998; Schaeffer and Weber, 1999; Schlessinger, 2000].

Among the proteinases involved in vascular remodeling secondary to injury, the matrix metalloproteinases (MMPs) play a prominent role. The members of the MMP family of proteinases can collectively degrade all major protein components of the ECM and have been implicated in a variety of tissue remodeling processes [Mignatti and Rifkin, 2000]. MMP-3 (stromelysin-1) is of particular interest because of its broad substrate specificity and tissue distribution. MMP-3 degrades collagen types III, IV, and V, laminin, fibronectin, elastin, and proteoglycans, and is secreted by a variety of cell types including vascular smooth muscle (SMC) and EC [Henney et al., 1991; Mignatti and Rifkin, 2000]. High levels of MMP-3 have been described in atherosclerotic plaques [Galis et al., 1994] and in the SMC of atherosclerotic coronary arteries [Henney et al., 1991]. In addition, a common mutation in the MMP-3-promoter that causes reduced gene expression is associated with progression of coronary atherosclerosis [Ye et al., 1996], indicating that MMP-3 expression by vascular cells has an important role in atherogenesis.

A variety of growth factors and cytokines, including FGF-2, platelet-derived growth factors (PDGFs), interleukin-1 alpha (IL-1α), tumor necrosis factor alpha (TNFα), and transforming growth factor beta-1 (TGFβ-1) regulate MMP-3 expression in several cell types [Kerr et al., 1990; Hanemaaijer et al., 1993; Galis et al., 1994; Rawdanowicz et al., 1994; Mignatti and Rifkin, 2000; Shimazu and Morishita, 2003]. Most of these growth factors and cytokines control MMP expression in a variety of cells through activation of mitogen-activated protein kinase (MAPK) family members [Westermarck and Kahari, 1999; Shimazu and Morishita, 2003]. However, the mechanisms that control MMP-3 expression in vascular EC have not been characterized. Because FGF-2 regulates a number of proteolytic activities in EC [Mignatti and Rifkin, 2000], we tested its effect on the expression of MMP-3 in these cells. obtained from FGF-2$^{-/-}$ mice showed an enhanced response to exogenous FGF-2, and therefore were chosen for this study. Here we report that FGF-2 upregulates EC expression of MMP-3 with a unique mechanism determined by the time-course of activation of the ERK-1/2 pathway.

MATERIALS AND METHODS

Materials

Human recombinant FGF-2 (hrFGF-2) was a generous gift from Scios Nova (Sunnyvale, CA) or was purchased from Gibco BRL, Life Technologies, Inc. (Rockville, MD). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Cells and Culture Conditions

Murine EC were isolated from both FGF-2$^{-/-}$ and wild-type mice as described [Pintucci et al., 2002], and cultured in αMEM (Gibco BRL, Life Technologies, Inc.) containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, Inc.). Cells between the 5th and 15th passage in culture were used for the experiments described.

Cell Treatments and Preparation of Cell-Conditioned Medium

Confluent EC were serum-starved (0.5% FCS) overnight and incubated with or without the indicated concentrations of hrFGF-2 from 0 to 24 h in medium containing 0.5% FCS. In some experiments, the cells were preincubated for 30 min with either 0.1% dimethylsulfoxide (DMSO; vehicle) or 50 μmol/L of the MAPK kinase (MEK)-1/2 inhibitor PD98059 (Calbiochem, San Diego, CA), or 10 μmol/L of the MEK-1/2 inhibitor UO126 (Promega Corporation, Madison, WI), or with 1 μmol/L of the phosphatydil-inositol 3-kinase (PI3-K) inhibitor wortmannin. Alternatively, UO126 was added to the culture medium at different times before or after the addition of hrFGF-2, as indicated. In other experiments, the cells were preincubated (30 min) with 2 μg/ml of cycloheximide (CHX) prior to hrFGF-2 addition. Cell-conditioned medium was harvested on ice, centrifuged at 14,000 rpm for 5 min in an Eppendorf centrifuge, and either analyzed immediately or stored at -20°C.

Characterization of Signaling Pathway Activation

At the indicated times after addition of hrFGF-2, the cells were washed with ice-cold PBS and scraped on ice in lysis buffer (50 mmol/L of HEPES, pH 7.5, 150 mmol/L of NaCl,

1 mmol/L of EDTA, 10% glycerol, 1% Triton-X-100, 25 mmol/L of sodium fluoride) containing 10 μg/ml of leupeptin (Roche, Indianapolis, IN), 1 mmol/L of Pefabloc (Roche), and 1 mmol/L of sodium ortho-vanadate. Following centrifugation in a refrigerated Eppendorf centrifuge (14,000 rpm, 5 min), the lysates were assayed for protein content by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL) using bovine serum albumin (BSA) as a standard. Cell extract protein (30–100 μg) was electrophoresed in SDS/10% polyacrylamide gels. The proteins were transferred onto PVDF membranes (Millipore, Bedford, MA) that were incubated with rabbit anti-phospho- ERK-1/2 or anti-phospho-Akt antibodies (Cell Signaling Technologies, Beverly, MA). The membranes were then incubated with horseradish peroxidase-linked anti-rabbit IgG antibodies (Amersham Pharmacia Biotech, Piscataway, NJ). Antigen-antibody complexes were detected by enhanced chemiluminescence (ECL LumiLight, Roche). Subsequently, the membranes were stripped of the antibodies by agitation in stripping solution (62.5 mmol/L of Tris-HCl, pH 7.5, 2% SDS, 100 mmol/L of β-mercaptoethanol)) for 1 h at 37°C, followed by 2 washes with Tris buffered saline, pH 7.8, (TBS) containing 0.05% Tween-20, and two washes with TBS alone. Membranes were then incubated with rabbit anti-total ERK-2 or anti-total Akt antibodies (Santa Cruz, Santa Cruz, CA), respectively, to control for equal loading and transfer of the samples.

Western Blotting

Aliquots (30–50 μl) of cell-conditioned medium were run in a 10% SDS–PAGE. Proteins were transferred onto a PVDF membrane that was incubated with rabbit anti-human MMP-3 antibody (Chemicon, Temecula, CA) and subsequently with horseradish peroxidase-linked anti-rabbit IgG antibody (Amersham Pharmacia Biotech, Inc.). Antigen-antibody complexes were detected as described above.

Casein Zymography

Aliquots (5 μl) of cell-conditioned medium were run in a Novex 12% Zymogram (Casein) gel (Invitrogen, Carlsbad, CA). The gel was washed in renaturing buffer (Invitrogen) and incubated in developing solution (Invitrogen) overnight at 37°C. Caseinolytic bands were evidenced after fixation/staining with 45% (v/v) methanol/10% (v/v) acetic acid/0.5% (w/v) Coomassie Brilliant Blue R-250 and destaining with methanol/acetic acid.

Immunoprecipitation

A 150 μl of medium conditioned by cells treated with 10 ng/ml of hrFGF-2 for 24 h was incubated with either 5 μg of anti-MMP-3 antibody or isotype-matched non-immune rabbit IgG in the presence of 0.5% (w/v) SDS for 30 min at 4°C. Immunocomplexes were precipitated by addition of 25 μl of Protein A-agarose (Roche) (50% slurry) and rocking for 30 min at 4°C. Equal amounts of the supernatants were loaded onto a casein gel as described above.

Northern Blotting

Total RNA was extracted from the cells with Trizol reagent (Gibco BRL, Life Technologies, Inc.) according to the manufacturer's instructions. The RNA (5–20 μg) was run in a 1% formaldehyde-agarose gel and transferred to a positively charged Nytran membrane (Schleicher and Scheull, Keene, NH). The membrane was prehybridized with ExpressHyb solution (Clontech, Palo Alto, CA) followed by hybridization with a ^{32}P-dCTP-labeled or biotinylated 308 bp rat MMP-3 cDNA probe obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) with the primers described [Webb et al., 1997].

RESULTS

FGF-2 Induces EC Expression of MMP-3 Through Activation of the ERK-1/2 Pathway

To study the role of FGF-2 in EC expression of MMP-3 mouse EC were treated with a saturating concentration (10 ng/ml) of hrFGF-2. Addition of the growth factor to the culture medium upregulated MMP-3 protein and mRNA expression. This effect was much stronger with EC derived from mice genetically deficient in FGF-2 (FGF-2$^{-/-}$) than with wt EC (Fig. 1A). For this reason, all subsequent experiments were performed with FGF-2$^{-/-}$ cells. The following results were reproduced in cultured FGF-2$^{-/-}$ cells throughout the number of passages indicated in "Materials and Methods."

The effect of hrFGF-2 (10 ng/ml) on MMP-3 mRNA and protein levels was time-dependent (Fig. 1A,B). Because the Ras/ERK and PI-3K/Akt pathways mediate most FGF-2 effects

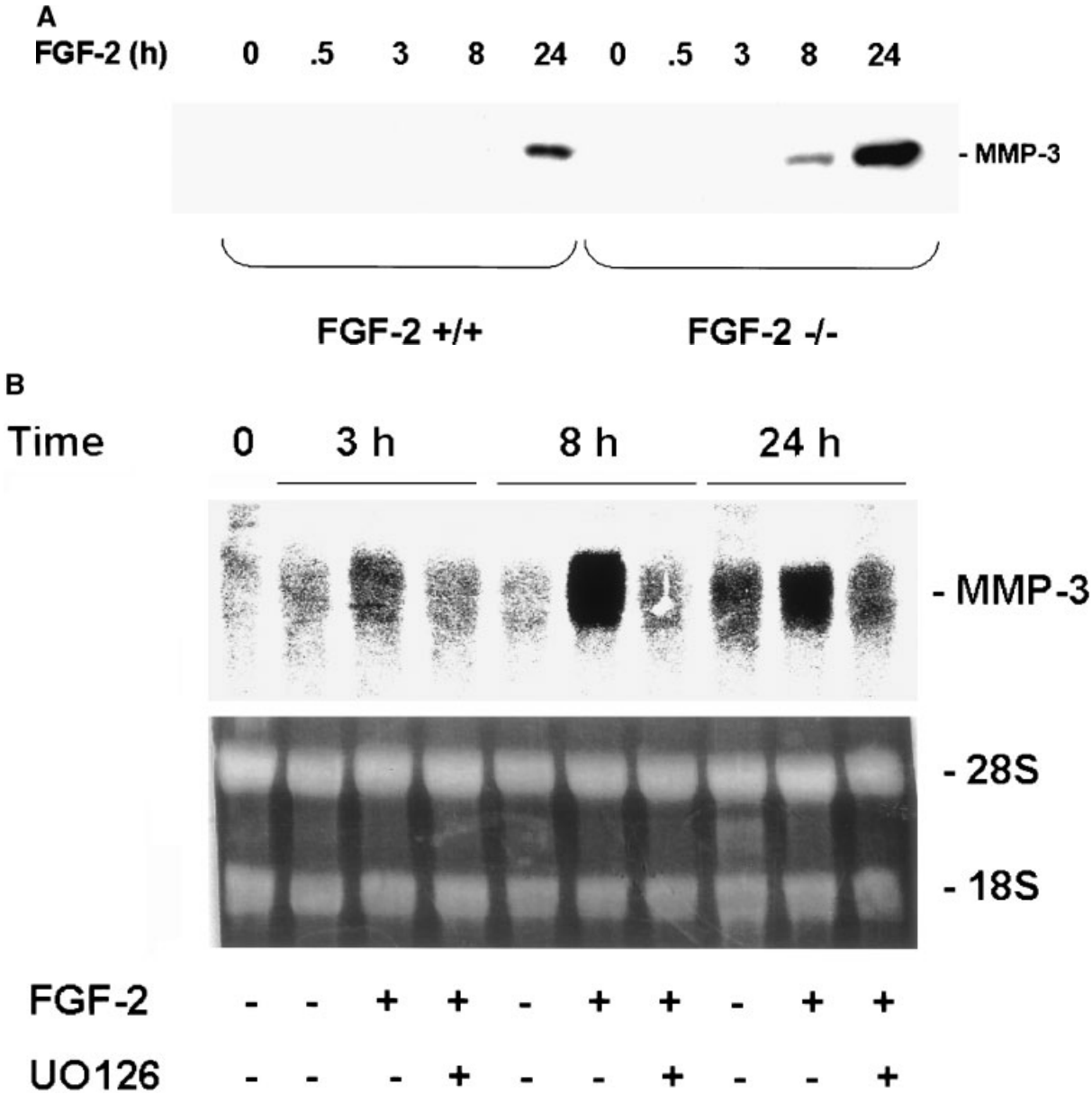

Fig. 1. FGF-2 upregulates MMP-3 expression in FGF-$2^{-/-}$ and wt endothelial cells (EC). **A**: Western blotting analysis of MMP-3 in the conditioned medium of wt (FGF-$2^{+/+}$) or FGF-2 deficient (FGF-$2^{-/-}$) EC incubated for the indicated times in the presence of 10 ng/ml of hrFGF-2. The protein blot was incubated with anti-human MMP-3 antibody, and immunoreactive bands were detected as described in ''Materials and Methods.'' A single band with an apparent M of ~55 kDa, consistent with the expected M of MMP-3, was detected. This experiment was repeated three times with comparable results. **B**: Northern blotting analysis of total RNA from FGF-$2^{-/-}$ EC incubated for the indicated times with either control medium or with medium supplemented with 10 ng/ml of hrFGF-2 in the presence or absence of 10 μmol/L of UO126. The RNA blot was hybridized with a ^{32}P-dCTP-labeled cDNA probe to human MMP-3 as described in ''Materials and Methods.'' Ribosomal RNA is shown as a control in the lower panel. This experiment was repeated three times with similar results.

on a variety of cells [Bikfalvi et al., 1997; Schlessinger, 2000], we targeted these pathways by means of specific inhibitors to characterize the mechanism(s) by which FGF-2 controls MMP-3 expression in EC. Inhibition of the PI-3K pathway with wortmannin did not affect MMP-3 production by hrFGF-2-treated cells. In contrast, two inhibitors of ERK-1/2 activation, PD98059 and UO126, blocked FGF-2 induction of MMP-3 mRNA and protein expression (Figs. 1C and 2A). Both PD98059 and UO126 inhibited ERK-1/2 activation; however, UO126 had a more potent and longer-lasting effect than PD98059 (Fig. 2B). In addition,

UO126 also upregulated FGF-2-induced Akt phosphorylation (Fig. 2C). These results indicated that the effect of FGF-2 on EC expression of MMP-3 is mediated by the ERK-1/2 pathway.

FGF-2-Induced Upregulation of MMP-3 mRNA Requires Protein Synthesis

Northern blotting analysis showed that hrFGF-2 treatment of EC resulted in an increase of steady-state MMP-3 mRNA levels, which peaked 8 h after addition of the growth factor (Fig. 1B). This finding suggested that the effect of FGF-2 might be mediated by the synthesis of intermediate protein(s). To test this hypothesis, EC were treated with hrFGF-2 in the presence or absence of cycloheximide (CHX), a protein synthesis inhibitor, and MMP-3 expression was characterized by Northern blotting. CHX completely abolished FGF-2-induced MMP-3 mRNA upregulation (data not shown), showing that the effect of FGF-2 requires protein synthesis.

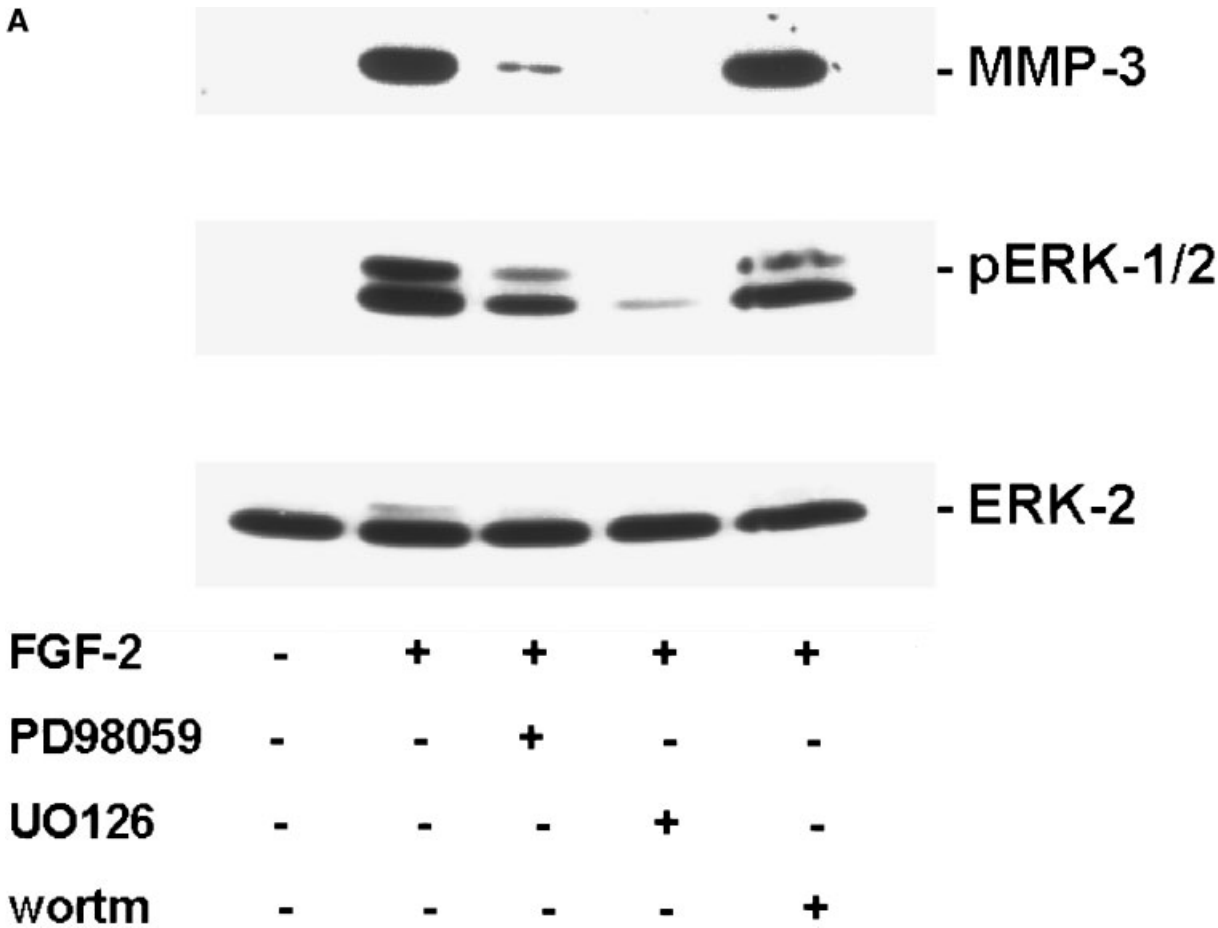

Fig. 2. Effect of FGF-2 on EC expression of MMP-3 and ERK-1/2 activation. **A**: FGF-$2^{-/-}$ EC were incubated for 24 h in the presence or absence of 10 ng/ml of hrFGF-2 and the indicated MEK-1/2 (PD98059, UO126) or PI-3K (wortm = wortmannin) inhibitors. DMSO (vehicle) was used as a control. The conditioned medium was analyzed by Western blotting with antibodies to MMP-3 .The lysates corresponding to cells treated similarly but harvested 30 min after treatments were characterized by Western blotting with antibodies to active ERK-1/2 (pERK-1/2) or total ERK-2 (ERK-2) as described in ''Materials and Methods.'' This experiment was repeated three times with comparable results. **B**: Western blotting analysis of ERK-1/2 activation in FGF-$2^{-/-}$ EC incubated for the indicated times in the presence or absence of 10 ng/ml of hrFGF-2 and the MEK-1/2 inhibitors PD98059 or UO126. The cells were preincubated with the inhibitors or with DMSO (vehicle) as a control as described in ''Materials and Methods.'' Cell lysates were characterized for active ERK-1/2 (pERK-1/2) and total ERK-2 (ERK-2) as described above. This experiment was repeated three times with comparable results. **C**: Western blotting analysis of Akt activation (pAkt) in FGF-$2^{-/-}$ incubated for 30 min in the presence (+) or absence (−) of 10 ng/ml of hrFGF-2 and the indicated MEK-1/2 (UO126) or PI-3K (wortm = wortmannin) inhibitors. The cells were preincubated with the inhibitors or with DMSO as a control, and cell lysates were characterized for active Akt (pAkt) and total Akt (AkT) as described in ''Materials and Methods.'' This experiment was repeated twice with comparable results. Inhibitor concentrations: PD98059, 50 μmol/L; UO126, 10 μmol/L; wortmannin, 1 μmol/L.

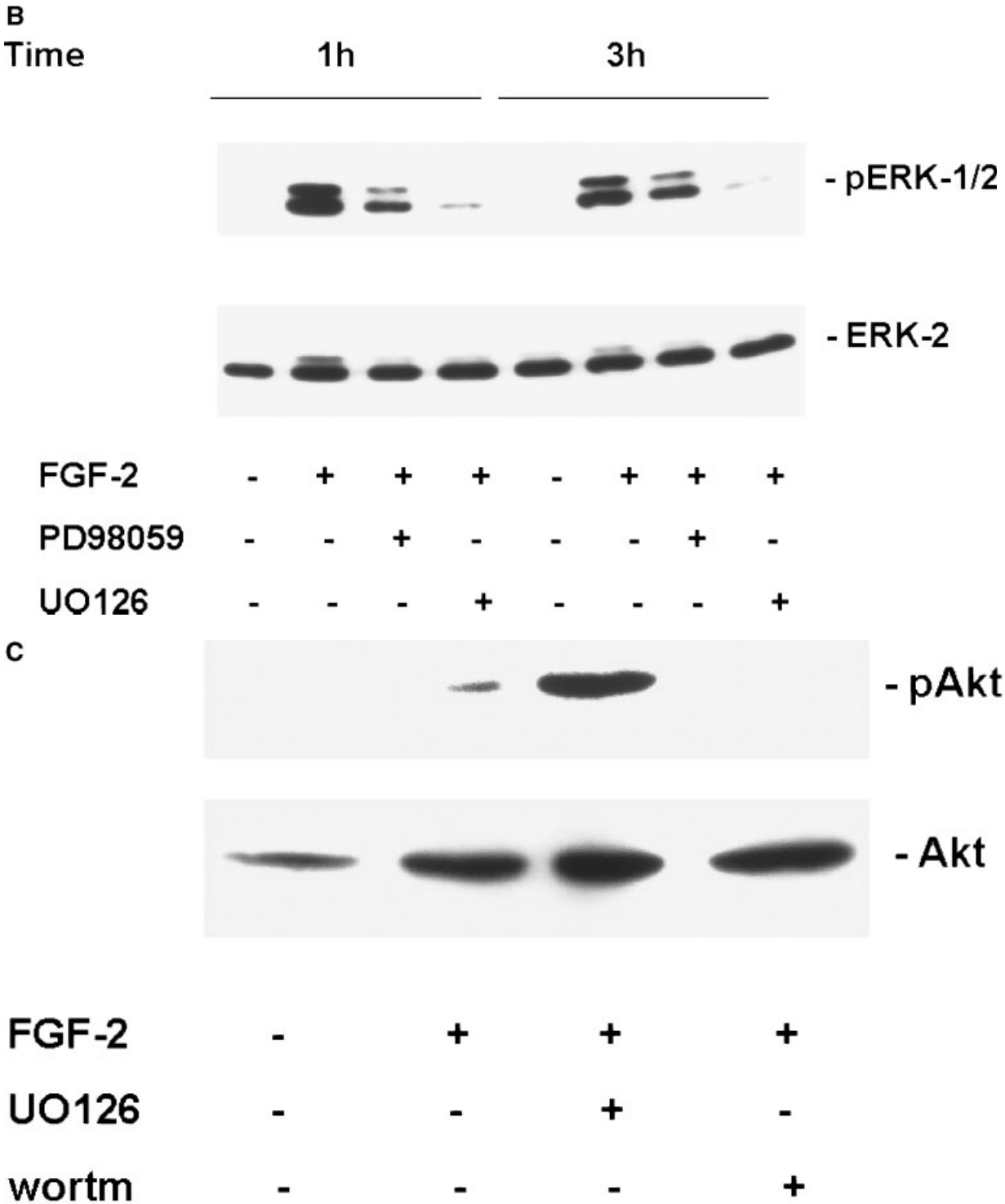

Fig. 2. (*Continued*)

FGF-2 Induction of MMP-3 Expression Requires Prolonged Activation of the ERK-1/2 Pathway

In preliminary experiments, both 1 ng/ml and 10 ng/ml of hrFGF-2 induced a rapid and strong activation of ERK-1/2; however, the lower concentration had a negligible effect on MMP-3 expression (data not shown). To clarify this apparent discrepancy, we analyzed the kinetics of ERK-1/2 activation in EC treated with either 1 ng/ml or 10 ng/ml of hrFGF-2. Both growth factor concentrations induced comparable levels

of ERK-1/2 activation at early time-points (30 min–3 h). However, in cells treated with 1 ng/ml of hrFGF-2 ERK-1/2 activation dramatically decreased after 8 h of incubation; in contrast, in cells treated with 10 ng/ml of hrFGF-2 significant levels of active ERK-1/2 were mantained for at least 24 h (data not shown).

In a second set of experiments, we tested ERK-1/2 activation and MMP-3 expression in cells treated with increasing concentrations (1, 2, 5, and 10 ng/ml) of hrFGF-2. The results showed that 1 ng/ml and 2 ng/ml of hrFGF-2 did not induce sustained (24 h) activation of ERK-1/2 and had a modest effect on MMP-3 expression. In contrast, 5 and 10 ng/ml both induced prolonged ERK-1/2 activation and strongly upregulated MMP-3 levels with a similar effect (Fig. 3). Consistent with the immunoblotting results, casein zymography showed that 5 and 10 ng/ml of hrFGF-2 also induced expression of a caseinolytic activity at the expected molecular mass of MMP-3. In contrast, lower concentrations were ineffective (Fig. 3) This caseinolytic activity was depleted from the conditioned medium after immunoprecipitation with anti-MMP-3 antibody (data not shown). These results indicated that sustained activation of the ERK-1/2 pathway is required to mediate FGF-2 induction of both MMP-3 expression and activity in mouse EC.

To test this hypothesis, EC were incubated with the MEK inhibitor UO126 for different times before or after treatment with 10 ng/ml of hrFGF-2. Inhibition of ERK-1/2 activation 30 min prior to, or 3 or 8 h after hrFGF-2 treatment blocked both ERK-1/2 activation and induction of MMP-3 expression (Fig. 4). Thus, FGF-2 upregulates MMP-3 expression and activity with a threshold dose–response mechanism determined by a requirement for sustained activation of ERK-1/2.

DISCUSSION

The data reported show that FGF-2 induces EC expression of MMP-3 through sustained activation of the ERK-1/2 pathway. This conclusion is supported by our findings that addition of exogenous FGF-2 upregulates MMP-3 expression and activity, and induces activation of the ERK-1/2 pathway; conversely, inhibition of ERK-1/2 activation blocks FGF-2-induced

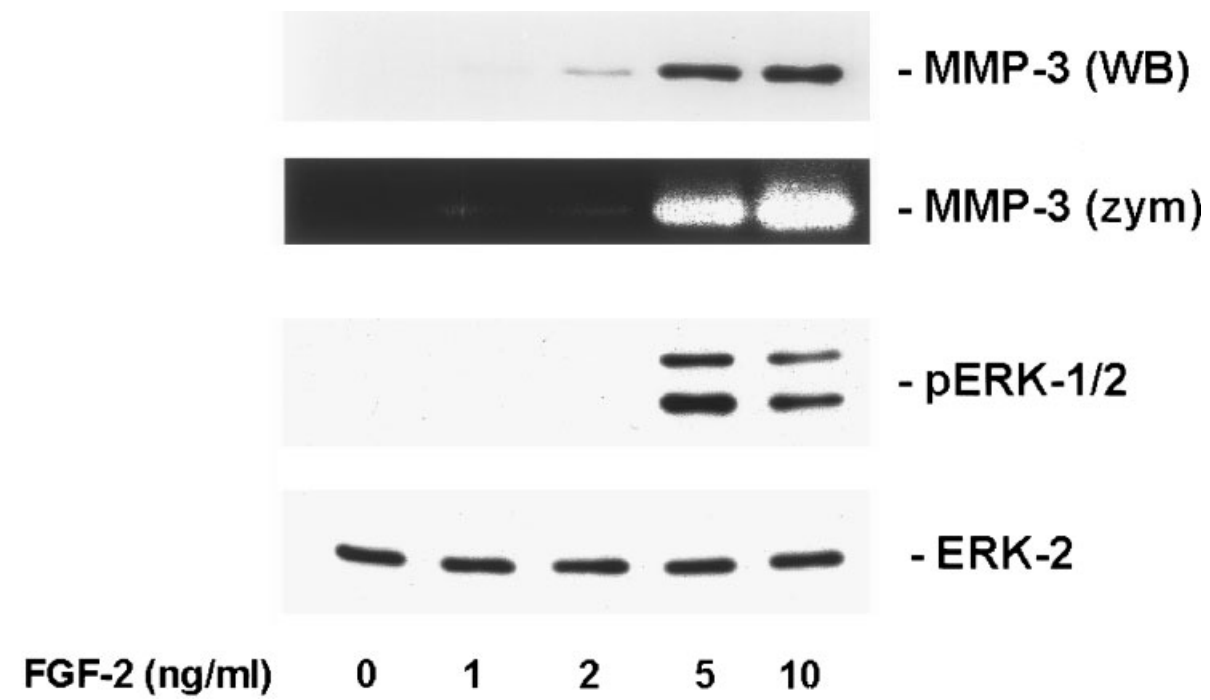

Fig. 3. Threshold effect of FGF-2 on EC expression of MMP-3. Medium conditioned by FGF-$2^{-/-}$ cells incubated with the indicated concentrations of FGF-2 for 24 h was analyzed by casein zymography (zym) and by Western blotting with antibodies to MMP-3 (WB). The corresponding cell lysates were analyzed by Western blotting with antibodies to active ERK-1/2 (pERK-1/2) or total ERK-2 (ERK-2) as described in ''Materials and Methods.'' This experiment was repeated three times with comparable results.

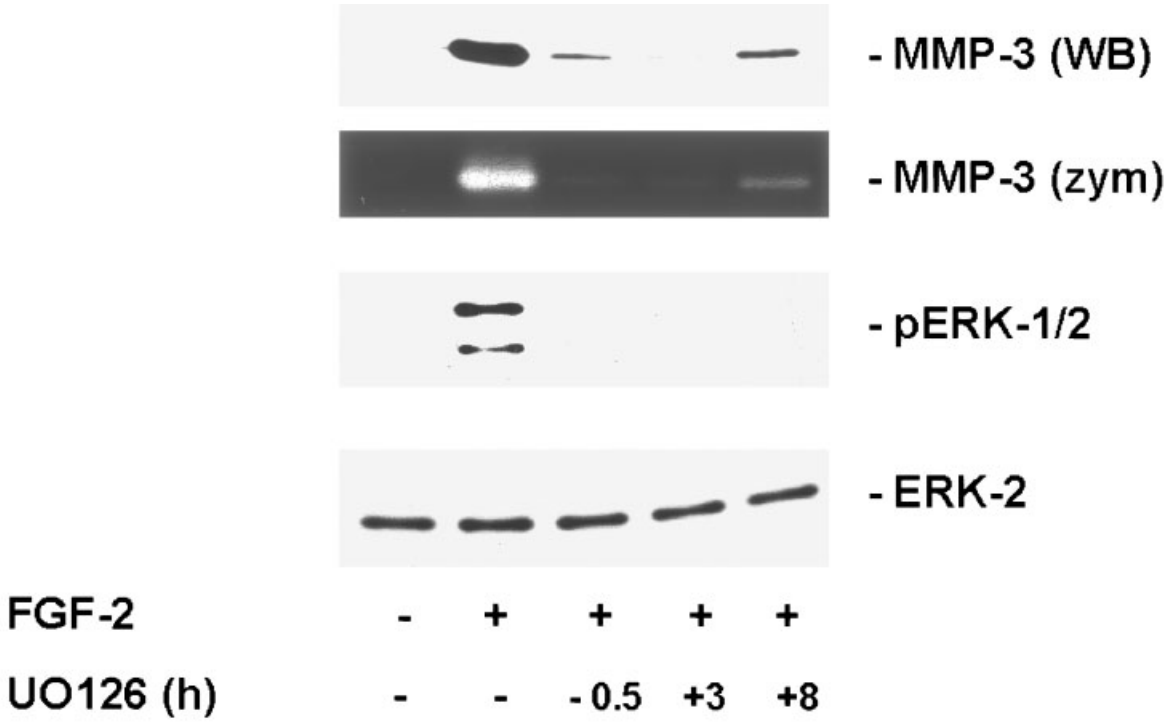

Fig. 4. Prolonged activation of ERK-1/2 is required for MMP-3 expression. FGF-$2^{-/-}$ EC were incubated for 24 h in the presence or absence of 10 ng/ml of hrFGF-2 with or without addition of the MEK inhibitor UO126 (10 mmol/L) at the indicated times prior to (−) or after (+) hrFGF-2 addition. DMSO (vehicle) was used as a control. The conditioned medium was analyzed by zym and by Western blotting with antibodies to MMP-3 (WB). The corresponding cell lysates were characterized by Western blotting with antibodies to active ERK-1/2 (pERK-1/2) or total ERK-2 (ERK-2) as described in ''Materials and Methods.'' This experiment was repeated three times with comparable results.

MMP-3 upregulation. Only FGF-2 concentrations that generate a prolonged activation of the ERK-1/2 pathway induce MMP-3 expression and activity; in contrast, a robust but transient activation of the ERK-1/2 pathway is scarcely effective.

We found that wt EC that produce FGF-2 do not express significant amounts of MMP-3, and that induction of MMP-3 expression by exogenous FGF-2 is higher in FGF-$2^{-/-}$ than in wt EC. Several non-mutually exclusive mechanisms can explain this apparent discrepancy. It is possible that either the amount of endogenous FGF-2 and/or its release in wt cells is inadequate to induce expression of detectable levels of MMP-3, or that acute stimulation of the ERK pathway is necessary for this effect. In addition, in wt cells the constitutive production of FGF-2 may generate an autocrine loop of FGF receptor (FGF-R) downregulation [Bikfalvi et al., 1995; Bikfalvi et al., 1997] that makes these cells less responsive to exogenous FGF-2 than FGF-$2^{-/-}$ cells. Consistent with the former hypothesis, our results show that MMP-3 expression is significantly induced only by hrFGF-2 concentrations equal to or higher than 5 ng/ml, lower concentrations being virtually ineffective. The second hypothesis is supported by the observation that overexpression of FGF-2 in transfected NIH 3T3 cells, which generates an autocrine loop of FGF-R activation [Bikfalvi et al., 1995], does not result in MAPK activation (Pintucci and Rifkin, unpublished results). The use of FGF-$2^{-/-}$ cells provides a unique tool to study the role of this growth factor without the interference of endogenous FGF-2.

The Ras/ERK and PI-3K/Akt pathways mediate most effects of FGF-2 on a variety of cells. We, therefore, targeted these signaling pathways with specific inhibitors. Wortmannin, a PI-3K inhibitor, did not affect FGF-2-induced expression of MMP-3 although it effectively inhibited Akt phosphorylation. In contrast, two inhibitors of ERK-1/2 activation, PD98059 and UO126, inhibited FGF-2-induced upregulation of MMP-3 expression. UO126 not only was a stronger inhibitor of ERK-1/2 activation but also had a longer-lasting inhibitory effect than PD98059. Because pretreatment of mouse EC with UO126 resulted in a more marked decrease

of MMP-3 expression as compared to the effect of PD98059 these data altogether indicated that the time course of ERK-1/2 activation plays an important role in the control of EC expression of MMP-3. This conclusion is further supported by the observation that transient activation of the ERK-1/2 pathway resulted in very poor induction of MMP-3 expression.

Activation of different intracellular signaling pathways has been shown to control phenotypic changes according to their integration and kinetics. Cowley et al. showed that the effect of factors that induce differentiation or proliferation of PC12 cells through the ERK pathway is determined by the kinetics of ERK activation [Cowley et al., 1994; Marshall, 1995]. Examples of this phenomenon are also illustrated by the mechanism(s) that control MMP expression in different cell lines. In the osteoblastic cell line MC3T3 FGF-2 decreases stromelysin-3 mRNA levels when given acutely, whereas prolonged exposure to the same growth factor results in stromelysin-3 upregulation [Delany and Canalis, 1998]. In the keratinocyte cell line SCC-12F epidermal growth factor (EGF) upregulates MMP-9 by inducing sustained activation of the ERK pathway [McCawley et al., 1999]. More recently, sustained ERK activation induced by FGF-2 in tumor cells expressing high levels of N-cadherin has been shown to be required for induction of MMP-9 expression [Suyama et al., 2002]. Prolonged activation of MAPK pathways has been hypothesized to modify profoundly the state of activation of certain transcription factors [Marshall, 1995]. The recent observation that sustained activation of ERK-1/2 stabilizes the transcription factor c-fos provides a molecular mechanism to this hypothesis [Murphy et al., 2002]. Consistent with these observations, our results show that FGF-2 induces MMP-3 expression and activity by activating the ERK-1/2 pathway rapidly and in a sustained manner, and that this effect requires de novo protein synthesis. In contrast, robust yet transient activation of ERK-1/2, either induced by low FGF-2 concentrations or resulting from pharmacological inhibition (+3 h, +8 h) of the signaling pathway, fails to induce significant upregulation of both the MMP-3 expression and activity.

Although the data presented here mostly refer to FGF-2 deficient mouse microvascular EC, our finding that EC expression of MMP-3 is regulated by FGF-2 may be relevant to understanding the mechanism(s) that control vascular remodeling, as both MMP-3 and FGF-2 levels are modulated during this process [Lindner and Reidy, 1991; Schwartz et al., 1995; Lijnen et al., 1999; Lijnen, 2001]. FGF-2-induced activation of ERK-1/2 is a key mechanism of endothelial wound healing in vitro. Vascular EC from FGF-$2^{-/-}$ mice lack both ERK-1/2 activation and the capacity to migrate in response to wounding. Addition of FGF-2 to the culture medium restores both ERK-1/2 activation and normal cell migration [Pintucci et al., 2002]. Whereas the role of FGF-2-induced MMP-3 expression in EC migration remains to be established, a consistent body of experimental evidence has shown the role of several MMPs in vascular remodeling. Targeting MMPs either with synthetic inhibitors or by increasing expression of their physiological inhibitors TIMP-1 or TIMP-2 decrease intimal hyperplasia in a variety of experimental models [Forough et al., 1996; Zempo et al., 1996; Cheng et al., 1998; Dollery et al., 1999]. Gene therapy modulation of vascular MMP expression prior to vein grafting to the arterial system effectively reduces intimal hyperplasia and is a promising molecular tool to control proteolysis in situ without systemic effects [Fernandez et al., 1998; George et al., 1998a,b, 2000]. Oral administration of all-trans retinoic acid (ATRA) also decreases both MMP-2 expression and intimal hyperplasia in experimental vein interposition grafts [Leville et al., 2000]. In this light, the observation that retinoids can modulate EC production of FGF-2 [Gaetano et al., 2001] indicates a further link between this growth factor and MMP expression in vascular cells.

The requirement for prolonged ERK-1/2 activation by FGF-2 points to a unique control mechanism for MMP-3 expression in vascular EC. A variety of EC functions, including urokinase plasminogen activator (uPA) and uPA receptor (uPAR) expression are controlled by FGF-2 in a linear dose-responsive manner [Mignatti et al., 1991a; Mignatti and Rifkin, 1996]. In contrast, relatively high concentrations of FGF-2 are required to induce MMP-3 expression with a threshold effect. This observation suggests that the role of MMP-3 in vascular remodeling is different from that of other proteinases, and that the level of this enzyme in vessels must be tightly controlled. High MMP-3 levels may be required when high amounts of FGF-2 are made available to the

tissue. Because FGF-2 is not efficiently secreted [Mignatti et al., 1991b; Bikfalvi et al., 1995], high concentrations of FGF-2 in the extracellular environment can derive from lethal or sublethal damage of the cell membrane or from cell lysis [Lindner and Reidy, 1991; Mignatti and Rifkin, 1991; Muthukrishnan et al., 1991; Clarke et al., 1993]. This will in turn upregulate an array of factors, including MMP-3, implicated in paracrine and autocrine EC response to stress and injury. The intensity and/or persistence of these phenomena are of particular importance in determining the outcome of vascular remodeling.

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