Insulin augments matrix metalloproteinase-9 expression in monocytes

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Abstract

Objective: Insulin resistance and hyperinsulinemia are major causes of cardiovascular morbidity and mortality. Matrix metalloproteinases (MMPs), highly expressed in activated mononuclear cells in vulnerable atherosclerotic lesions, are the main proteolytic enzymes controlling plaque stability. The aim of this study was to investigate the regulation of monocyte MMP-9 by insulin.

Methods and results: Stimulation of MMP-9 expression by insulin was time- and concentration-dependent in human monocytic THP-1 cells. Inhibition of insulin receptor (IR) maturation via inhibition of its activating convertase furin with the pharmacological furin-inhibitor decanoyl-RVKR-chloromethylketone, as well as blocking of IGF-1R function with a IGF-1R blocking antibody, demonstrated that insulin mediates increases in MMP-9 via IR activation. Inhibition of insulin's "metabolic" phosphatidylinositol 3-kinase signaling with wortmannin (50 nmol/L) or LY294002 (2.5 μmol/L) did not prevent insulin-dependent MMP-9 induction. In contrast inhibition of insulin's "mitogenic" Ras-Raf-mitogen-activated protein kinase–kinase pathways with PD98059 (15 μmol/L) or U0126 (2 μmol/L) inhibited insulin-induced MMP-9 gelatinolytic activity in THP-1 cells. Likewise, PD98059 inhibited insulin augmented MMP-9 levels in primary human monocytes, whereas wortmannin had no effect.

Conclusion: This study demonstrates that insulin can induce MMP-9 via mitogenic signaling pathways in monocytes, whereas phosphatidylinositol 3-kinase-dependent signaling, typically altered in insulin resistance, is not required. Induction of MMP-9 by insulin may potentially contribute to a pro-inflammatory state and the increased cardiovascular morbidity and mortality in type 2 diabetics.
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Insulin resistance and hyperinsulinemia, which cluster with obesity, dyslipidemia and hypertension in the metabolic syndrome, are a major cause of premature cardiovascular morbidity and mortality [1]. Atherosclerotic plaque rupture, causally related to the majority of acute coronary syndromes, commonly occurs at sites of continuous inflammation and collagen degradation [2]. Matrix metalloproteinases (MMPs) are the primary proteolytic enzymes in the extracellular space, contributing to weakening of the plaque cap via their ability to cleave the extracellular matrix (ECM) [3]. Rupture prone atherosclerotic lesions are characterized by the accumulation of activated mononuclear inflammatory cells (MNCs) and their increased expression of MMPs [4]. Basically the >26 mammalian MMPs can be grouped according to their mode of activation into soluble MMPs (e.g. MMP-2 and -9) and the membrane-bound MT-MMPs. Soluble MMPs are released as zymogens and then activated extracellularly by other MMPs or serine proteases such as plasmin [3]. In contrast MT-MMPs are activated in the *trans*-Golgi network by furin [5], then tethered to the plasma membrane as active enzymes, targeting their proteolytic activity to the pericellular space [3].

Clinical and experimental studies have implicated MMP-9 (gelatinase B) as a key determinant of atherosclerotic plaque stability [6–8]. MMP-9 principally derives from monocytes/macrophages [9,10], the major cell type involved in the initiation, progression and complications of atherosclerosis. This MMP-isozyme may regulate lesion stability via its capacity to cleave the ECM (e.g. collagen type IV, fibronectin

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or laminin), as well as "non-matrix" substrates, such as adhesion molecules and growth factors [11]. In MNCs MMP-9 is strongly inducible by a number of inflammatory mediators, including TNF-α and oxidized LDL [10,12]. Recently, studies have shown that MMP-9 serum levels are increased in patients with insulin resistance/type 2 diabetes [13,14], potentially contributing to their increased cardiovascular risk. However, a direct contribution of hyperinsulinemia to MMP-9 expression in MNCs is unknown. In this study we investigated the regulation of MNC MMP-9 by insulin and its involved signaling pathway.

1. Materials and methods

Cell culture medium and materials were from Invitrogen. The pharmacological MEK1 inhibitor PD98059, the MEK1/2 inhibitor U0126, its control U0124 and the p38 MAPK inhibitor SB203580 were from Calbiochem. The PI3-kinase inhibitors wortmannin, LY294002 and its control LY303511 were from Calbiochem; the mTOR/p70^{s6}-kinase inhibitor rapamycin and the antibody to actin were from Sigma. The furin-like proprotein convertase inhibitor decanoyl-RVKR-chloromethylketone (dec-CMK) was from Bachem. TNF-α was from TEBU (FRG) and angiotensin II and insulin were purchased from Sigma. The antibody to furin (MON152) was from Alexis Biochemicals and the antibody to the insulin receptor (IR) α-subunit was from Dianova. Antibodies to MMP-9 and TIMP-1 were purchased from Labvision (FRG). The antibody to the IGF-1Rβ-subunit (C-terminus), to the IRβ-subunit (C-terminus) and the monoclonal functional IGF-1Rα blocking antibody (clone 1H7) and its control isotype were from Santa Cruz. Levels of signaling pathway activation were detected with phospho-specific antibodies, recognizing pERK1/2 MAP-kinase, pAkt, (p)p70^{s6}-kinase, (p)p38, p-eNOS or p-GSK-3α/β and corresponding antibodies to non-phosphorylated forms purchased from Cell Signaling.

1.1. Cell culture

The human monocytic THP-1 cell line was obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany). Cells were cultured in RPMI 1640, supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin at 95% relative humidity and 5% CO$_2$ at 37 °C as described [10]. For experiments, cells were rendered quiescent by serum-starvation overnight. In inhibition experiments, cells were pre-treated with dec-CMK (12 h), pharmacological signaling pathway inhibitors (1 h) or the IGF-1R blocking antibody (1 h) in serum-free media, followed by stimulation in the presence of the inhibitors.

Primary human monocytes were obtained from healthy volunteers and purified as described by Cipolletta et al. [15]. Briefly, blood samples were collected in lithium heparin-containing tubes and processed within 1 h. CD14$^+$ mono-

cytes were isolated from blood samples using the MACS column technology and CD14 MicroBeads (Miltenyi Biotech.) as recommended by the manufacturer. High purity of monocytes was confirmed by flow cytometry (>95% CD14$^+$). Human monocytes were cultured in RPMI 1640, supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin at 95% relative humidity and 5% CO$_2$ at 37 °C for 6–8 h, then synchronized overnight and used in pharmacological inhibitor experiments, as described above for THP-1 cells. Viability of THP-1 monocytes and primary human monocytes was monitored by propidium iodide analysis and trypan blue exclusion. Experiments were done in triplicates, at the least.

1.2. Immunoblotting

Western blot analysis has been described recently [10]. Briefly, proteins were extracted in a buffer (20 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 2.5 mmol/L Na-pyrophosphate, 1 mmol/L Na$_3$VO$_4$) containing freshly dissolved protease inhibitors (Complete EDTA-free, Boehringer). Up to 50 µg of proteins was subject to 10% reducing SDS-PAGE.

1.3. Gelatin zymography

Conditioned medium was mixed 1:1 with Novex buffer (Invitrogen) and electrophoresed in 10% SDS-PAGE containing 0.1% gelatin. Gels were renaturated by exchanging SDS for Triton X-100 (2.5%), followed by 24 h incubation at 37 °C in activation buffer (50 mmol/L Tris, pH 7.6; 5 mmol/L CaCl$_2$; 0.2 mol/L NaCl and 0.02% Brij). Gels were subsequently stained with Coomassie staining solution (0.5% Coomassie R250; 30% MeOH; 10% acetic acid) for 2 h, followed by destaining (50% MeOH and 10% acetic acid). Supernatants from the human fibrosarcoma cell line (HT1080) were used as standard.

1.4. Flow cytometry analysis

Monocytic THP-1 cells (200,000 cells/sample) were washed once with PBS–0.2% NaN$_3$–0.15% bovine serum albumin (BSA)–1 mg/mL hIgG (500 ×g, 5 min, 5 °C), giving a final volume of 300 µL. After the addition of 2.5 µg/sample control antibody (polyclonal rabbit IgG [Sigma] or mouse IgG1 [#2010, DIATEC]) or specific antibodies (polyclonal rabbit–anti-furin convertase [MON152; #ALX-803-017-R100, Alexis] or monoclonal mouse–anti-insulin receptor antibody [IRα; #DLN-10220, Dianova]) samples were incubated for 30 min at 5 °C and washed again. Then 7.5 µg/sample secondary antibody goatF(ab′)2-anti-rabbit IgG (H+L)-FITC (#FI-1000, VECTOR) or goat–anti-mouse IgG (H+L)-FITC (#115-096-062, Jackson Immunotec) was added and incubated in the dark for 30 min at 5 °C. Cells were then washed as above and the fluorescence measured with the FACScan (BD Biosciences, USA) in the

fluorescence-1 channel (20,000 events/sample). The fluorescence histograms were generated using the WinMDI2.8 software after gating for THP-1 cells in the FSC/SSC dot plot.

1.5. Data analysis

Semiquantitative densitometry was done using the NIH program 1.62 and is expressed in arbitrary units (A.U.). Data are expressed as mean±SD. Groups were compared using 1-way ANOVA followed by Bonferroni/Dunn multiple comparison test. Statistical significance was designated at a probability value of $p < 0.05$.

2. Results

2.1. Insulin induces MMP-9 gelatinolytic activity in human monocytic cells

THP-1 monocytes were rendered quiescent by serum-starvation overnight and then stimulated with 10 nmol/L insulin for 24 h. In comparison experiments, cells were stimulated with 10 μmol/L angiotensin II (Ang II) or 10 ng/mL TNF-α, a potent inducer of MMP-9 [10]. Compared to controls, insulin and TNF-α strongly increased pro-MMP-9 gelatinolytic activity and protein levels, whereas Ang II had no effect (Fig. 1A, B, C). Increases in pro-MMP-9 were paralleled by an induction of tissue inhibitor of matrix metalloproteinases (TIMP)-1, which typically accompanies MMP-9 synthesis/release in MNCs [11]. Neither insulin nor TNF-α or Ang II had any effect on constitutively expressed pro-MMP-2 (Fig. 1A). In vivo, MMP-9 is primary released as zymogen, which is then activated extracellularly by other proteases [11]. Thus, we next investigated whether insulin-mediated increases in pro-MMP-9 can be activated to the fully active MMP-9 by 4-aminophenylmercuric acetate (APMA). Compared to unstimulated cells, APMA treatment (1 mmol/L, 4 h) of conditioned medium from insulin stimulated THP-1 cells resulted in a heightened generation of the mature 82 kDa MMP-9 from its 92 kDa precursor (Fig. 1D). Further analysis demonstrated that insulin increases pro-MMP-9 in dependence on the concentration (significant increase at 0.1 nmol/L insulin, 24 h stimulation; Fig. 2A and B; *$p < 0.05$ vs. controls). To ensure maximal stimulation, a concentration of 10 nmol/L insulin was used in further experiments. Time course studies revealed a significant increase of MMP-9 gelatinolytic activity following 12 h stimulation with 10 nmol/L (Fig. 2C and D; *$p < 0.05$ vs. controls).

2.2. Regulation of THP-1 monocyte MMP-9 by insulin involves insulin receptor activation

To dissect the role of insulin receptor (IR) signaling in insulin-induced pro-MMP-9, we investigated IR involvement by inhibition of IR activation. The mature, membrane-expressed IR derives from endoproteolytic cleavage of its propeptide in the *trans*-Golgi network (TGN) by furin [16]. FACS analysis demonstrated significant expression of IR and its activating convertase furin on monocytic THP-1 cells (Fig. 3A and B; full line = control (isotype), dotted line = specific antibody). Inhibition of furin with the specific inhibitor dec-CMK [17] (50 μmol/L) inhibited IR activation, evident by the increase of the 200 kDa IR TGN pro-form and a simultaneous decrease of the mature 100 kDa IR (Fig. 3C). In agreement with the inhibition of IR maturation, insulin-mediated increases of MMP-9 gelatinolytic activity were abolished in dec-CMK treated cells (Fig. 3D). In contrast,
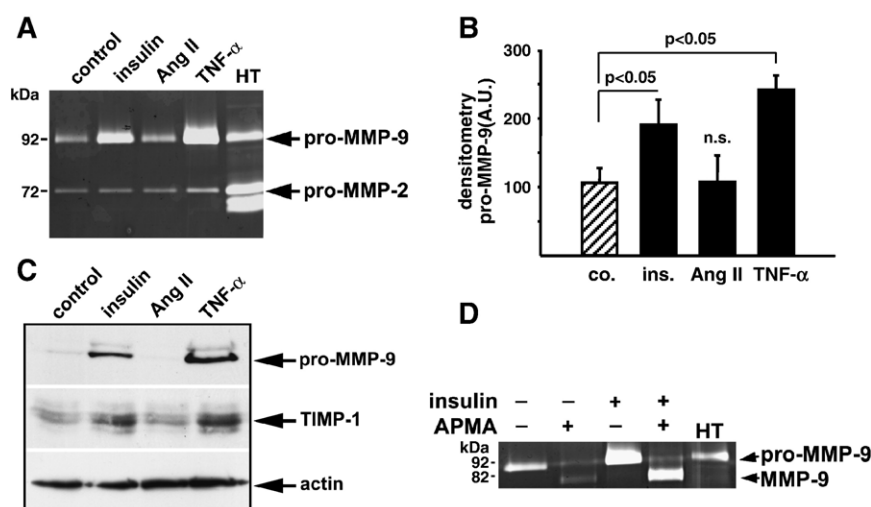

Fig. 1. (A) THP-1 monocytes were stimulated with insulin (10 nmol/L), angiotensin II (Ang II; 10 μmol/L) or TNF-α (10 ng/mL) for 24 h and conditioned media analyzed by zymography. Insulin and TNF-α significantly increased pro-MMP-9, whereas Ang II had no effect and neither insulin nor TNF-α or Ang II affected pro-MMP-2. Densitometry of pro-MMP-9 is depicted in B ($n = 5$). (C) Insulin (10 nmol/L) and TNF-α (10 ng/mL) induced protein levels of pro-MMP-9 and TIMP-1 (membrane reblotted with actin; $n = 3$). (D) Insulin-induced pro-MMP-9 (92 kDa) is convertible to its fully active 82 kDa form by incubation of supernatants with 4-aminophenylmercuric acetate (APMA; 1 mmol/L, 4 h; co. = control; $n = 3$). HT = HT1080.
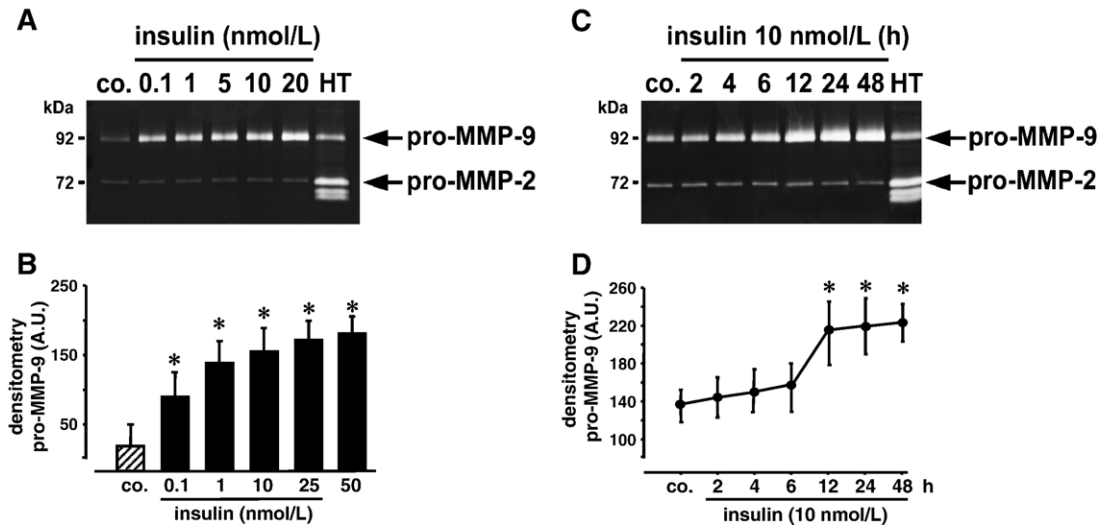
Fig. 2. Pro-MMP-9 induction by insulin was concentration-dependent, with a significant increase at 0.1 nmol/L insulin stimulation (A and B; *$p<0.05$ vs. controls (co.); 24 h stimulation; $n=6$). Pro-MMP-9 increased time-dependently and was significantly induced following 12 h stimulation with 10 nmol/L insulin (C and D; *$p<0.05$ vs. controls (co.); $n=6$). HT = HT1080.

inhibition of furin did not prevent TNF-α (10 ng/mL) induced pro-MMP-9, because the TNF receptor does not require endoproteolytic activation by furin. We further investigated IR involvement by blocking IGF-1R function using a specific monoclonal IGF-1R blocking antibody. Because IGF-1 is a relatively weak inducer of MAP-kinases [18], Akt phosphorylation was investigated to asses antibody functionality. Blocking IGF-1R activation with an IGF-1R blocking antibody (clone 1H7; 5 μg/mL) repressed IGF-1 (50 ng/mL)-dependent Akt phosphorylation, but did not significantly affect insulin (10 nmol/L)-mediated Akt activation, confirming specificity of the antibody to the
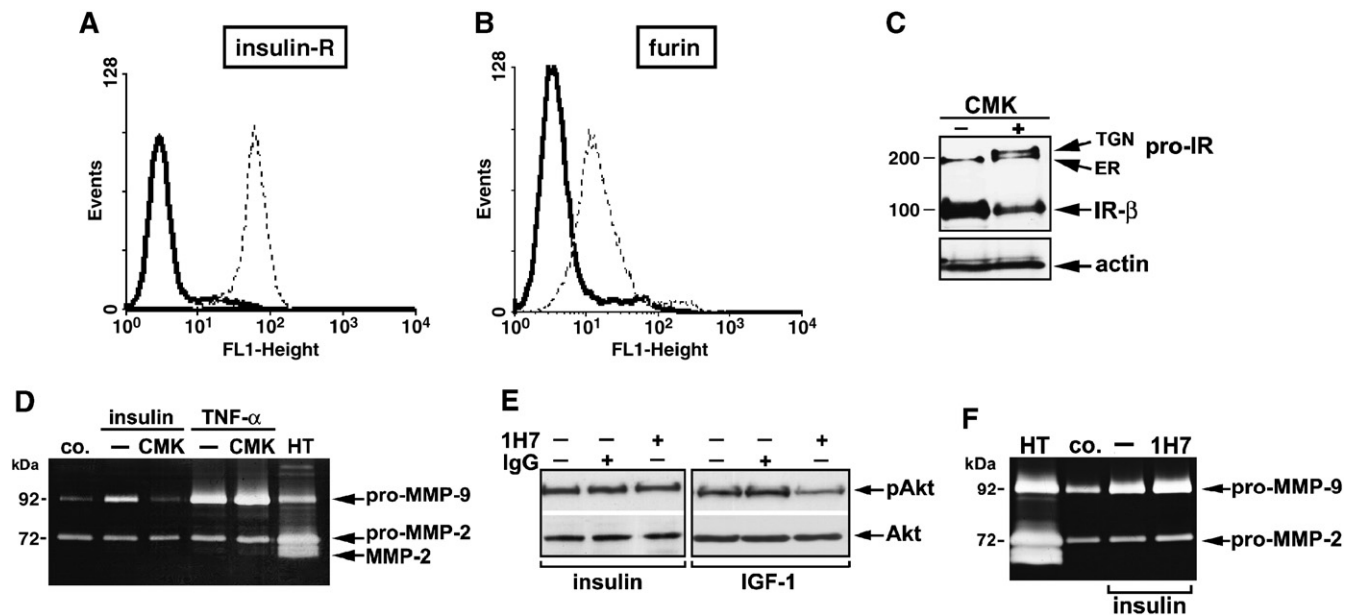

Fig. 3. (A and B) FACS analysis demonstrated that THP-1 monocytes express the IR and its activating convertase furin (full line = control isotype; dotted line = specific antibody; $n=3$). (C) Inhibition of furin in THP-1 cells with dec-CMK (50 μmol/L) inhibited endoproteolytic activation of the IR, evident by the increase of its 200 kDa *trans*-Golgi network (TGN) form (ER = endoplasmic reticulum) and concurrent decease of the mature 100 kDa IR (membrane reblotted with actin; $n=3$). (D) Inhibition of IR activation with dec-CMK abolished insulin (10 nmol/L; 12 h)-mediated pro-MMP-9 induction, but had no effect on TNF-α (10 ng/mL; 12 h) induced pro-MMP-9 ($n=4$). (E) A IGR-1R blocking antibody was used to further dissect IR/IGF-1R involvement. The IGF-1R blocking antibody (clone 1H7; 5 μg/mL) prevented IGF-1 (50 ng/mL; 20 min) dependent Akt phosphorylation, but had no significant effect on insulin (10 nmol/L; 20 min)-mediated Akt activation, demonstrating specificity of the antibody for the IGF1-R. An isotype control had no effect ($n=3$). (F) Accordingly, 1H7 had no effect on insulin (10 nmol/L, 12 h)-induced pro-MMP-9 in THP-1 monocytes, supporting the hypothesis that insulin mediates pro-MMP-9 induction primary via IR activation ($n=3$). HT = HT1080.
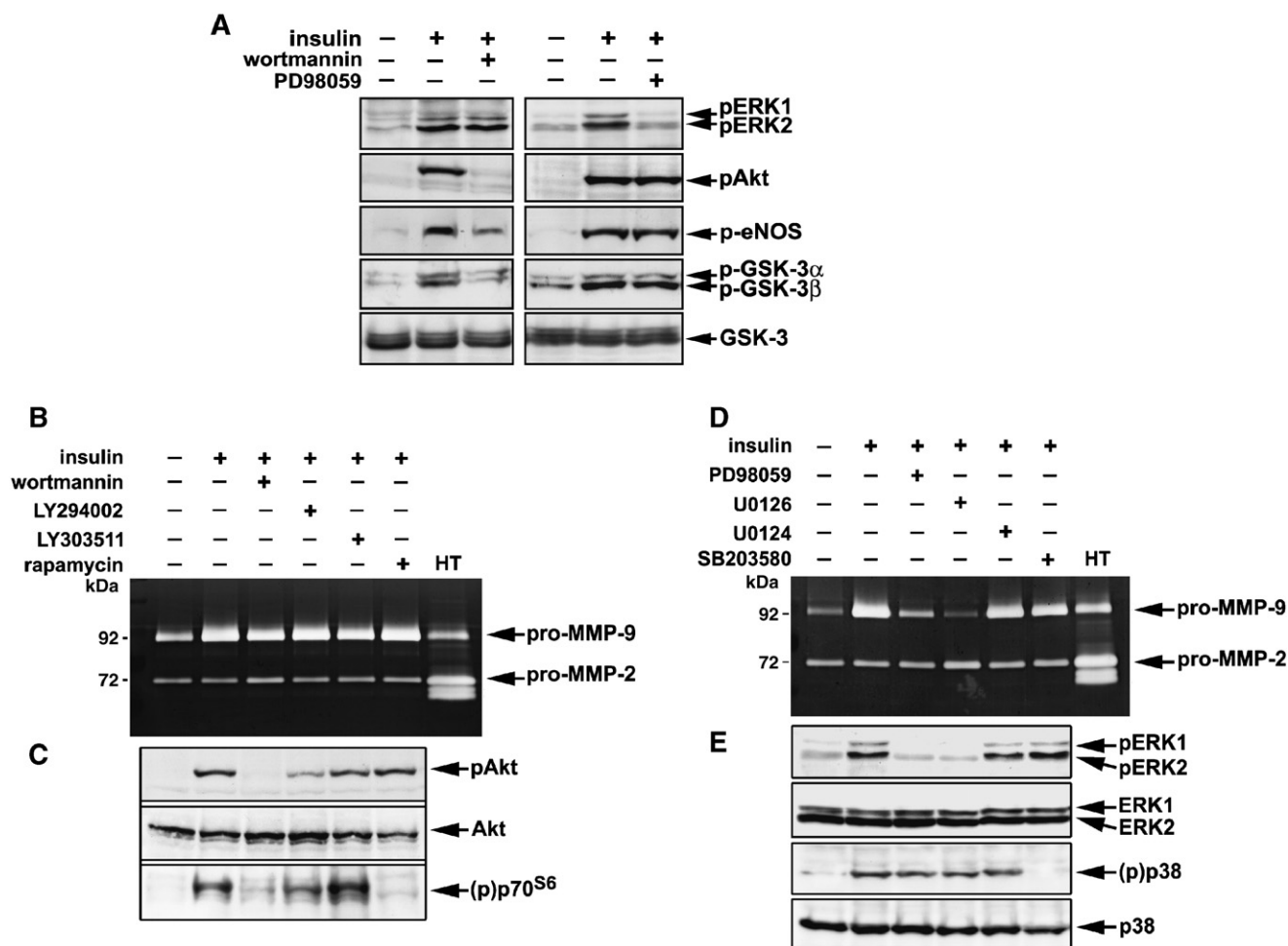
Fig. 4. (A) Insulin (10 nmol/L; 20 min) induces the "metabolic" PI3-kinase pathway (Akt, eNOS, GSK-3α/β) and the "mitogenic" ERK1/2 MAP-kinase pathway in THP-1 monocytes. The "metabolic" pathway is inhibited by wortmannin (50 nmol/L), whereas the "mitogenic" pathway is inhibited by the MEK1 inhibitor PD98059 (15 μmol/L) in THP-1 monocytes ($n=3$). (B) Inhibition of PI3-kinase-dependent pathways with wortmannin (50 nmol/L) or LY294002 (2.5 μmol/L) did not affect insulin (10 nmol/L; 12 h)-induced pro-MMP-9. LY303511 (2.5 μmol/L) was used as inactive control. Rapamycin (100 ng/mL), an inhibitor of mTOR/p70^{s6}-kinase signaling, did not inhibit insulin-mediated pro-MMP-9 either. Inhibition of PI3-kinase signaling pathways by the respective pharmacological inhibitors is depicted in (C) ($n=4$). (D) In contrast, the MEK1 inhibitor PD98059 (15 μmol/L) and the MEK1/2 inhibitor U0126 (2 μmol/L) significantly inhibited insulin (10 nmol/L; 12 h)-induced MMP-9, whereas the MEK1/2 control U0124 (2 μmol/L) and the p38 MAP-kinase inhibitor SB20350 (2.5 μmol/L) had no effect. Successful inhibition of the ERK1/2 MAP-kinase signaling pathways is depicted in E ($n=4$). HT = HT1080.

IGF-1R (Fig. 3E). An isotype control antibody had no effect on IGF-1 or insulin-dependent Akt activation. Consequently, inhibition of IGF-1R activation by the specific IGF-1R blocking antibody did not prevent insulin-induced pro-MMP-9 in monocytes, demonstrating that insulin mediates increases in MMP-9 gelatinolytic activity principally via IR activation (Fig. 3F).

2.3. Insulin induces MMP-9 in monocytes via MAP-kinase-dependent signaling

Pharmacological inhibitors were used to investigate the signaling pathways involved in insulin-mediated pro-MMP-9 induction in THP-1 monocytes. Immunoblotting with phospho-specific antibodies demonstrated that wortmannin (50 nmol/L), a specific PI3-kinase inhibitor, prevented insulin-mediated phosphorylation of protein kinase B (Akt), nitric oxide synthase (eNOS) and glycogen synthetase kinase (GSK)-3α/β, but had no effect on MAP-kinase activation (Fig. 4A). In contrast, the selective MEK1 inhibitor PD98059 (15 μmol/L) inhibited activation of ERK-1/2 MAP-kinases, but had no effect on insulin-dependent PI3-kinase→Akt signaling (Fig. 4A). Inhibition of the PI3-kinase pathway with wortmannin (50 nmol/L) or LY294002 (2.5 μmol/L), as well as the inhibitor control LY303551 (2.5 μmol/L) had no significant effect on insulin-induced MMP-9 gelatinolytic activity. Likewise inhibition of mTOR/p70^{s6}-kinase, a pathway downstream of PI3-kinase in insulin's "metabolic" signaling [19] with rapamycin (100 ng/mL), did not affect insulin-induced MMP-9 (Fig. 4B and C). In contrast, inhibition of MAP-kinases, either with the MEK1 inhibitor PD98059 (15 μmol/L) or the MEK1/2 inhibitor U0126 (2 μmol/L) significantly inhibited insulin-dependent increases of MMP-9, whereas the MEK1/

A

insulin   − + + +
wortmannin − − − +
PD98059   − − + −   HT
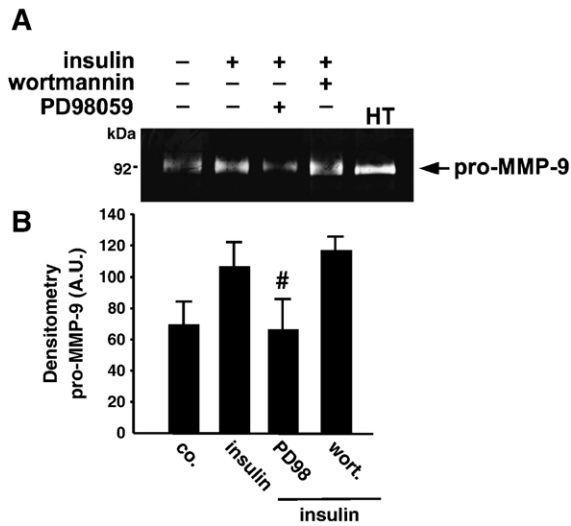
kDa
92— [gel image] ← pro-MMP-9

B

Fig. 5. (A) Insulin augmented MMP-9 gelatinolytic activity in primary human monocytes, obtained from healthy volunteers. Insulin-induced (10 nmol/L; 12 h) MMP-9 gelatinolytic activity was significantly inhibited with the MEK1 inhibitor PD98059 (PD98; 15 μmol/L), whereas wortmannin (wort.; 50 nmol/L) had no significant effect (densitometry depicted in B, #$p < 0.05$ vs. controls (co.); $n = 4$). HT = HT1080.

2 inhibitor control U0124 (2 μmol/L) or the p38 MAP-kinase inhibitor SB203580 (2.5 μmol/L) had no effect (Fig. 4D and E). Comparable results were obtained with primary human monocytes, in which insulin (10 nmol/L; 12 h) augmented pro-MMP-9 was significantly inhibited by the MEK1 inhibitor PD98059 (15 μmol/L), whereas the PI3-kinase inhibitor wortmannin (50 nmol/L) had no significant effect (Fig. 5A and B; #$p < 0.05$ vs. controls).

3. Discussion

Insulin resistance, the major characteristic of type 2 diabetes, is defined as the inability of insulin to facilitate glucose uptake and metabolism in target organs such as muscle and adipose tissue. Initially hyperinsulinemia, a state strongly associated with increased cardiovascular morbidity and mortality, compensates [20–22]. However, a direct contribution of hyperinsulinemia to the increased cardiovascular risk in type 2 diabetes is still debated, since beneficial and potentially harmful effects of insulin have been reported. Thus, insulin has been shown to inhibit apoptosis in THP-1 monocytes [23] and to induce NO-synthesis in endothelial cells (ECs) [24], but it can also induce plasminogen activator inhibitor type 1 (PAI-1) in ECs [25] or facilitate vascular smooth muscle cells (VSMCs) migration [26], whereby it may promote atherothrombosis and restenosis.

Here we demonstrate that insulin is a potent inducer of MMP-9 gelatinolytic activity in human monocytic THP-1 cells and primary human monocytes. This MMP-isozyme is highly expressed in activated macrophages of rupture prone atherosclerotic plaques [2,4] and serum levels of MMP-9 are increased in patients with acute coronary syndromes [7] or in stent restenosis [27]. Recently, in vivo experiments demon-

strated that forced overexpression of MMP-9 in macrophages induces plaque disruption in *apo*E−/− mice [6], supporting its role as a key regulator in the equilibrium of matrix synthesis/degradation, essential to vessel wall integrity. Interestingly, it has been shown that levels of MMP-9 are also significantly increased in type 2 diabetics [13,14], potentially contributing to the increased cardiovascular risk in these patients. Treatment of insulin resistance, either with intensive lifestyle modifications [28] or with the antidiabetic PPARγ-activators [13,14], has been demonstrated to reduce MMP-9 serum levels in patients with insulin resistance/type 2 diabetes.

Our study demonstrates, that insulin directly elicits a proinflammatory response in monocytes at concentrations recognized in patients with insulin resistance/type 2 diabetes [29]. Insulin mediates its "metabolic" and "mitogenic" effects principally via two distinct signaling pathways: the PI3-kinase pathway is responsible for glucose uptake, lipogenesis and glycogen synthesis, whereas insulin stimulated Ras-Raf-mitogen-activated protein kinase–kinase (MEK-signaling) mediates insulin's "mitogenic" actions [30]. In insulin resistance a selective post-receptor defect involving impairment of the beneficial "metabolic" PI3-kinase pathway, with an unaltered "mitogenic" MAP-kinase pathway, has been documented in the vasculature [31] and macrophages [32] of insulin resistant/diabetic animal models, as well as in skeletal muscle biopsies from insulin resistant patients [33]. Furthermore in the vasculature of insulin resistant animals, strong increases of ERK activation and concurrent decreases in Akt phosphorylation have been demonstrated in response to stent injury, potentially reflecting the altered tissue repair that accompanies the shift in proliferative/metabolic signaling balances in insulin resistance [34]. In vitro, impaired PI3-kinase but intact MAP-kinase signaling has been demonstrated to facilitate insulin-mediated VSMC and EC migration [26,35].

Here we show that, despite the inhibition of insulin's "metabolic" PI3-kinase/Akt/GSK-3 signaling, insulin can still induce MMP-9 gelatinolytic activity in monocytic THP-1 cells and primary human monocytes, whereas inhibition of insulin's "mitogenic" ERK1/2 MAP-kinase abolishes it. Nevertheless in hyperinsulinemic states, insulin may also bind to the IGF-1R [36], which shares >85% homology with the IR [37]. Activation of the IGF-1/IGF-1R axis is capable of upregulating MT1-MMP/MMP-2 activities itself, as we have recently demonstrated in VSMCs [18]. Thus to further explore IR/IGF-1R contribution in insulin-dependent induction of monocyte MMP-9, we initially targeted IR maturation via inhibition of its activating convertase furin. Furin, which has been shown to activate the pro-IR precursor in the TGN [16], is a member of the subtilisin/kexin-like proprotein convertases [38] which activate proproteins with narrow specificity following basic R-*Xaa*-K/R-R-like motifs [39]. Our study demonstrates that inhibition of IR maturation significantly inhibited insulin-induced MMP-9. However, furin also activates the IGF-1R in VSMCs [18] and THP-1

monocytes (data not shown); thus we further excluded binding of insulin to the IGF-1R using a specific IGF-1R blocking antibody. The IGF1-1R blocking antibody inhibited IGF-1-mediated IGF-1R activation, but had no effect on insulin-dependent IR activation and accordingly did not abolish insulin-dependent increases in MMP-9 gelatinolytic activity. This demonstrates that insulin can activate MMP-9 via its IR, independent of the IGF-1R in monocytes.

In conclusion, we demonstrate that insulin induces MMP-9 gelatinolytic activity and protein levels via its "mitogenic" ERK1/2 signaling pathways in monocytes, whereas PI3-kinase signaling, typically altered in insulin resistance, is not required. Therefore, induction of MMP-9 by insulin may potentially contribute to a pro-inflammatory state and the increased cardiovascular morbidity and mortality found in patients with insulin resistance and type 2 diabetes.

Acknowledgments

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