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Angiotensin II induces MMP-2 in a p47phox-dependent manner

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Abstract

Activated matrix metalloproteinases (MMPs) in patients with acute coronary syndromes may contribute to plaque destabilization. Since reactive oxygen species (ROS) induce MMP-2 and angiotensin II (ANG II) enhances NAD(P)H-oxidase-dependent ROS formation, we assessed whether ANG II induces MMP-2 in a NAD(P)H-oxidase-dependent manner. MMP-2 mRNA expression and activity were analyzed in wildtype and p47phox-deficient ($p47phox^{-/-}$) murine smooth muscle cells (SMC). To address a clinical implication, sections of human atherosclerotic arteries were stained for MMP-2, p47phox, ANG II, AT_1-receptor, and α-smooth muscle cell actin (α-SMC actin). MMP-2 protein expression and activity from these arteries were compared to those without atherosclerosis. ANG II enhances mRNA synthesis and activity of MMP-2 in a p47phox-dependent manner. Immunohistochemical analyses revealed a co-localization of MMP-2 with p47phox, ANG II, AT_1-receptor, and α-SMC actin. MMP-2 protein expression and gelatinolytic activity are increased in atherosclerotic arteries. Thus, activation of the renin-angiotensin system may contribute to plaque destabilization via ROS-dependent induction of MMP-2.

Keywords: Angiotensin II; MMP-2; NAD(P)H-oxidase; Atherosclerosis

Proteolytic imbalance determines arterial remodeling and plaque destabilization in atherosclerotic vessels. Dynamic processes, e.g., local cytokine expression, oxidative or hemodynamic stress, regulate collagen synthesis and breakdown, resulting in a dissolution of collagenous matrix in the plaque's fibrous cap due to high expression levels of matrix metalloproteinases (MMPs) [1]. The balance between MMPs and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) is thereby shifted towards increased proteolysis causing MMP-dependent degradation of the extracellular matrix (ECM). This latter weakens the plaque's structure and enhances the susceptibility of the fibrous cap to rupture when exposed to hemodynamic stress.

MMPs are endopeptidases which are classified according to their substrate specificity, e.g., gelatinases such as MMP-2 and MMP-9 [1]. Immunohistochemical studies revealed that MMPs are expressed by various cells present within atheromas. Especially MMP-2 is detectable in large amounts in macrophages, foam cells, and smooth muscle cells in atherosclerotic lesions [2].

Activity of MMP-2 is regulated at multiple levels such as gene transcription, protein synthesis, and post-translational activation of zymogens which can be induced by various stimuli. Moreover, recent evidence showed that reactive oxygen species (ROS) may enhance MMP-2 expression and activity [3–6]. In this regard, Van Wart and colleagues speculated that ROS are able

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to interact with the thiol-group, involved in preserving MMP-2 latency [7], thereby leading to MMP-2 activation. In fact, recent evidence suggests that ROS may enhance the activity of MMPs [8,5], especially that of MMP-2 [4]. Vasoactive peptides such as angiotensin II (ANG II) not only enhance hemodynamic stress of the vessel wall by mediating vasoconstriction but also stimulate ROS formation via the activation of the vascular NAD(P)H oxidase in a p47phox-dependent manner in vivo [9], and in vitro [10]. In addition, it was previously shown that ANG II and MMP-2 are co-localized in atherosclerotic plaques [11], suggesting their functional interaction. However, the role of the NAD(P)H oxidase as the major source of ROS in the vessel wall, in the ANG II-induced MMP-2 activation remains to be determined. To address this question, smooth muscle cells (SMC) isolated from mice aortas lacking the p47phox cytosolic subunit of the NAD(P)H-oxidase (p47phox$^{-/-}$), responsible for ROS formation, were analyzed following ANG II stimulation. To address a potential clinical implication of the in vitro studies, we analyzed the co-localization of components of the renin-angiotensin system (RAS) with MMP-2 and p47phox in human atherosclerotic left anterior descending (LAD) coronary artery sections from patients with ischemic cardiomyopathy (ICM).

The effector peptide of the RAS, ANG II, is mainly involved in the pathogenesis of atherosclerosis [12] and MMPs contribute to the progression of atherosclerotic plaque formation via enhanced extracellular matrix decomposition. Therefore, we investigated, whether MMP-2 protein expression and gelatinolytic activity are enhanced in atherosclerotic coronary arteries from patients with ICM compared to LAD coronary arteries from patients with dilated cardiomyopathy (DCM), who do not suffer from atherosclerotic plaques.

Materials and methods

Cell culture medium and supplements were from Gibco-BRL/Invitrogen, the monoclonal anti-human MMP-2 antibody (clone 42-5D11) was from Calbiochem, and the polyclonal AT_1-receptor antibody was from Santa Cruz Biotech. The polyclonal ANG II antibody was a generous gift from Jörg Nussberger [13], while the monoclonal α-smooth muscle actin antibody (clone 1A4) was from Sigma. The p47phox$^{-/-}$ mice were a generous gift from Steven Holland [22]. C57BL/6 mice were obtained from Jackson Laboratories. Primers were obtained from MWG, while the MMP-2 standard and ANG II were obtained from Sigma.

Cell culture. SMC from C57BL/6 mice (wild type, WT) and p47phox$^{-/-}$ mice backcrossed to the same genetic background were isolated and maintained as reported recently [4]. Cells were growth-arrested in DMEM containing 1% FCS for 24 h before stimulation with ANG II (10^{-7} mol/L).

RT-PCR. Total RNA from SMC stimulated with ANG II was isolated using TriFast-Reagent (peqLAB). Reverse transcription of mRNA and PCR were performed as described previously [4], with 24 cycles for [14] and 32 cycles for MMP-2 using a personal cycler (Biometra). The sequences of primer were as follows: 18S rRNA primer sequence forward 5′-GTA ACC CGT TGA ACC CCA TT-3′, reverse 5′-CCA TCC AAT CGG TAG TAG CG-3′, MMP-2 primer forward 5′-ATA CAG GAT CAT TGG TTA CAC ACC-3′, reverse 5′-GCT GCC ACG AGG AAT AGG-3′. PCR products were separated by 1% agarose gel electrophoresis and quantified densitometrically using a Gel Doc image analysis system (Bio-Rad, Hercules, CA).

Zymography. Forty microliters of supernatants from ANG II-stimulated SMC or 100 μg of coronary artery homogenates was analyzed by gelatin zymography as reported previously [4]. MMP-2 gelatinolytic activity was quantified densitometrically, using a Gel Doc image analysis system (Bio-Rad).

Tissue preparation. Samples of left anterior descending (LAD) coronary arteries were obtained from recipient hearts removed at transplant surgery from patients with ischemic cardiomyopathy (ICM, $N = 8$; age 55 ± 5 years) or with dilated cardiomyopathy (DCM, $N = 9$; age 43 ± 9 years). All patients received ACE-inhibitors, betablockers, and diuretics prior to transplantation. The tissue sampling procedure was approved by the Local Ethical Committee. For zymography and Western blot analysis, arteries were homogenized in ice-cold phosphate-buffered saline, supplemented with 0.1% Triton X-100, and centrifuged with 10,000*g* at 4 °C for 10 min.

Western blot analysis. Protein concentration was determined using Bradford Dye Reagent Concentrate (Bio-Rad). Sample buffer (125 mmol/L Tris, pH 6.8, 4% SDS, 10% glycerol, 0.006% bromophenol blue, and 2% β-mercaptoethanol) was added to tissue homogenates. The proteins were separated by 10% SDS–PAGE, transferred to PVDF membranes, and probed with anti-MMP-2 (1:1000). Results were normalized to α-smooth muscle cell (SMC) actin (1:1000). Proteins were visualized by enhanced chemiluminescence and analyzed densitometrically [9].

Immunohistochemical analysis. Serial sections from LAD human coronary artery were embedded in OCT and cryo-conserved, 5 μm serial sections were used for immunohistochemistry. Endogenous peroxidase activity was blocked by immersion in 0.3% H_2O_2 and nonspecific binding of primary antibody was blocked by incubation with 4% species-appropriate normal serum (Vector laboratories). Arteries were stained for MMP-2 (1:25), p47phox (1:50), ANG II (1:100), AT_1-receptor (1:50), and α-SMC actin (1:1000) for 3 h at room temperature. The secondary antibody coupled to a streptavidin–biotin complex was applied (ABC Elite kit, Vector laboratories). The final reaction was visualized with diaminobenzidine (DAB chromogen + substrate, DAKO cytomation) or 3-amino-9-ethylcarbazole (AEC; DAKO cytomation). Unspecific goat or rat IgG was used as negative controls.

Statistical analysis. Results are given as means ± SEM of at least four independent experiments. Statistical analysis was performed by Student's t test. Comparisons of >2 measurements were assessed with ANOVA. A value of $p < 0.05$ was considered to be statistically significant.

Results

ANG II enhanced MMP-2 mRNA expression via p47phox

ANG II (10^{-7} mol/L) stimulation of SMC obtained from WT mice induced a significant increase in MMP-2 mRNA expression starting at 0.5 h (1.5 ± 0.1-fold, $p < 0.05$) and was further enhanced at 3 h (2.1 ± 0.1-fold, $p < 0.05$). The ANG II-induced increase in MMP-2 transcripts was completely abolished in SMC generated from p47phox$^{-/-}$ mice (Figs. 1A and B).

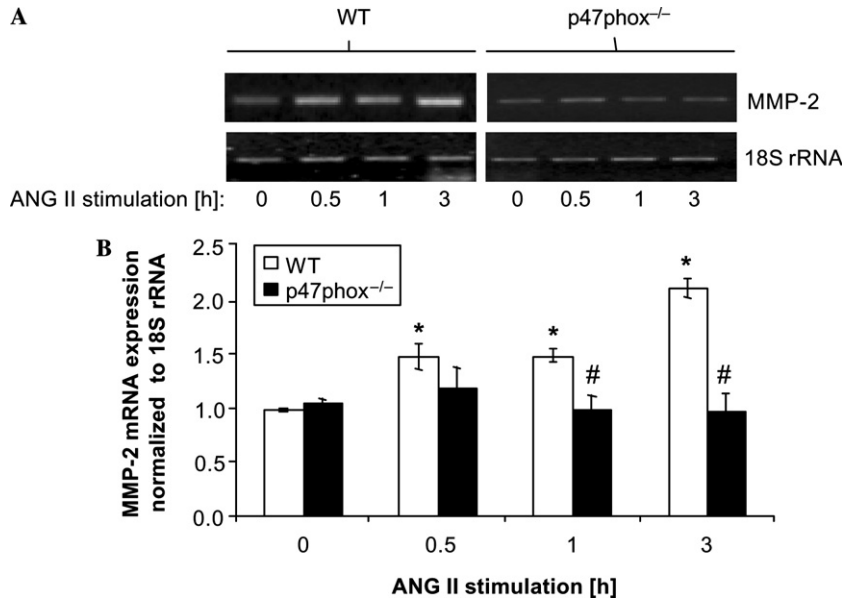

Fig. 1. (A) SMC from WT and p47phox$^{-/-}$ mice were stimulated with ANG II (10^{-7} mol/L) for 0, 0.5, 1, and 3 h and MMP-2 mRNA expression was investigated by RT-PCR. (B) Densitometric analysis of MMP-2 mRNA expression. Values were normalized to 18S rRNA. Data are given as means ± SEM of four independent experiments. *$p < 0.05$ vs. 0 h, $^{\#}p < 0.05$ vs. WT.

ANG II induced MMP-2 release in a p47phox-dependent manner

Murine aortal vascular SMC were found to secrete solely the latent form of MMP-2 (pro-MMP-2, 72 kDa). Moreover, no active form of MMP-2 (66 kDa) was detected by gelatin zymography (Fig. 2A). However, ANG II stimulation of SMC from WT mice induced a significant increase in gelatinolytic MMP-2 activity in the cell culture supernatants at 1 h (1.8 ± 0.2-fold, $p < 0.05$) and was further enhanced at 3 h (2.2 ± 0.3-fold; $p < 0.05$) reflecting an increased release of MMP-2. This increase of MMP-2 in response to ANG II was completely blunted in SMC from p47phox$^{-/-}$ mice. In addition, SMC from p47phox$^{-/-}$ mice were found to secrete significant lower levels of MMP-2 compared to SMC from WT mice (Figs. 2A and B).

Co-localization of MMP-2, p47phox, ANG II, and AT_1-receptor in human atherosclerotic plaques

In serial LAD sections of coronary arteries containing atherosclerotic plaques obtained from patients with

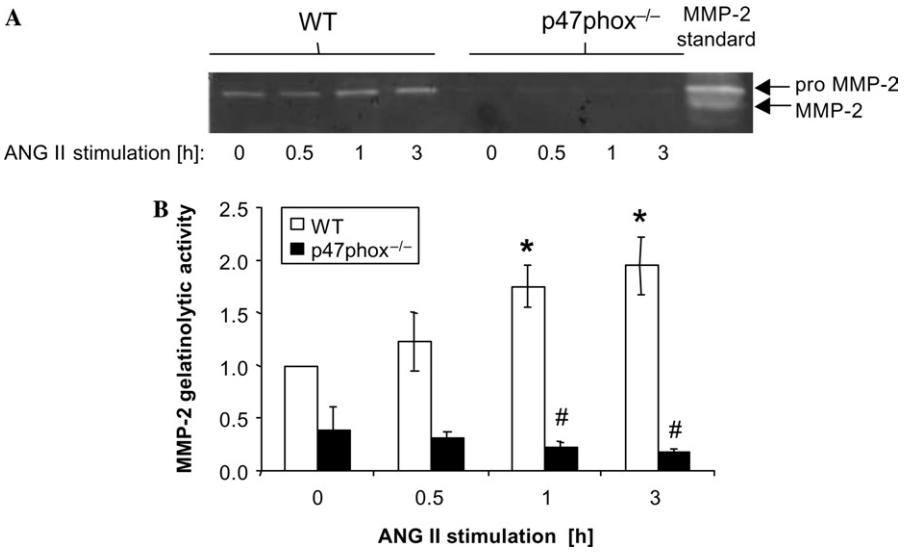

Fig. 2. (A) SMC from WT and p47phox$^{-/-}$ mice were stimulated with ANG II (10^{-7} mol/L) for 0, 0.5, 1, and 3 h and supernatants were subjected to gelatin zymography. (B) Densitometric analysis of gelatinolytic MMP-2 activity. Data are given as means ± SEM of four independent experiments. *$p < 0.05$ vs. 0 h, $^{\#}p < 0.05$ vs. WT.

ICM, α-SMC staining indicated the location of SMC mainly in the media (Fig. 3E). Parallel serial LAD sections of these patients stained for MMP-2 (Fig. 3A), p47phox (Fig. 3B), ANG II (Fig. 3C), and AT_1 receptor (Fig. 3D) revealed a co-localization of these components with the SMC-rich areas. In control sections the application of unspecific IgG as primary antibody revealed no specific staining (Fig. 3F).

Enhanced MMP-2 in human atherosclerotic coronary arteries

MMP-2 protein was investigated in extracts from atherosclerotic LAD coronary arteries from patients with ICM ($N = 8$) and compared to unaltered LAD coronary arteries from patients with DCM ($N = 9$) (Fig. 4). Both, MMP-2 protein expression determined by Western blot

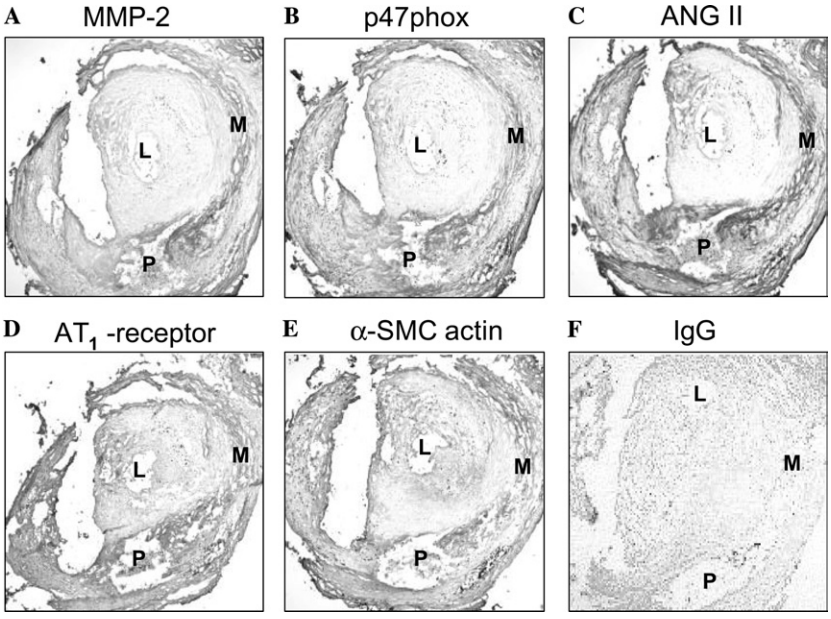

Fig. 3. In atherosclerotic left anterior descending (LAD) sections of coronary arteries from patients with ischemic cardiomyopathy (ICM) immunohistochemical staining was performed. In this figure, a typical staining for MMP-2 (A), p47phox (B), ANG II (C), AT_1-receptor (D), and α-SMC actin (E) is shown. An unspecific IgG antibody was used as control (F). M, media; P, plaque; and L, lumen. Magnification 40×.

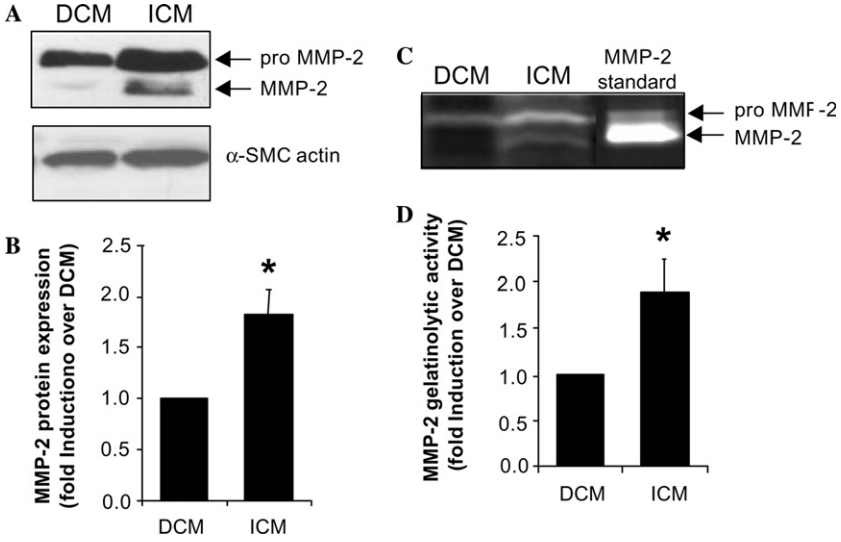

Fig. 4. Analysis of MMP-2 in atherosclerotic left anterior descending (LAD) of coronary arteries from patients with ischemic cardiomyopathy (ICM, $N = 8$) compared to LAD coronary arteries from patients with dilated cardiomyopathy (DCM, $N = 9$). (A) MMP-2 protein expression was investigated by Western blot analysis. (B) Densitometric analysis of MMP-2 protein expression. Values were normalized to α-SMC actin. $^*p < 0.05$ vs. DCM. (C) Gelatinolytic MMP-2 activity was investigated by zymography. (D) Densitometric analysis of MMP-2 gelatinolytic activity. $^*p < 0.05$ vs. DCM.

(Figs. 4A and B) and gelatinolytic MMP-2 activity (Figs. 4C and D) determined by zymography, were found to be enhanced in coronary arteries from patients with ICM (1.9 ± 0.3-fold, $p < 0.05$). In difference to our cell culture experiments with murine SMC we were able to detect the pro-form as well as the active form of MMP-2 in LAD coronary arteries from patients with ICM.

Discussion

The present study demonstrates that the ANG II-mediated increase in MMP-2 mRNA expression and protein release in murine SMC requires the p47phox containing NAD(P)H-oxidase system. In addition, immunohistochemical analysis revealed a co-localization of MMP-2 and p47phox with components of the RAS like ANG II and its receptor AT_1 in α-SMC actin-rich areas in human atherosclerotic plaques. Moreover, MMP-2 protein expression and gelatinolytic activity were found to be enhanced in atherosclerotic LAD coronary arteries from patients with ICM compared to non-atherosclerotic LAD coronary arteries from patients with DCM.

MMP-2 is produced in normal and atherosclerotic human vessels [1], especially in SMC [2]. Regulation of MMPs occurs at multiple levels: either by gene transcription and synthesis of inactive pro-enzymes, posttranslational activation of proenzymes, or via the interaction with their inhibitors (TIMP). Pro-inflammatory cytokines, e.g., interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), platelet derived growth factor (PDGF), phorbol ester [15], and enhanced oxidative stress, induce an increase in MMP-2 synthesis and activity, which could be correlated with cardiovascular events, e.g., myocardial infarction due to plaque rupture [5].

Like pro-inflammatory cytokines the activation of the AT_1-receptor is involved in the development and progression of atherosclerosis [16]. Since ANG II induces a NAD(P)H-oxidase-dependent ROS formation [10,9], we investigated whether ANG II induces MMP-2 mRNA expression in SMC in a NAD(P)H-oxidase-dependent manner. Here we report that ANG II induces an increase in MMP-2 mRNA expression which was blunted in p47phox$^{-/-}$ mice, suggesting an important role for the NAD(P)H-oxidase in the ANG II-dependent regulation of MMP-2 mRNA synthesis. Notably, the promoter of MMP-2 contains a functional transcription factor binding site for activating protein-1 (AP-1), which could be activated by ROS [17], potentially after ANG II-induced NAD(P)H-oxidase activation.

Besides transcriptional regulation, MMP-2 induction may also occur via enhanced secretion of its proenzyme. Therefore, we investigated whether ANG II induces MMP-2 release from SMC in a NAD(P)H-oxidase-dependent manner. Similar to the transcriptional regulation, we first demonstrate an ANG II-dependent increase of MMP-2 release which was completely blocked in p47phox$^{-/-}$ mice. In addition, we were able to show that SMC from p47phox$^{-/-}$ mice secrete significant lower levels of MMP-2. Therefore, it is tempting to speculate that the NAD(P)H-oxidase in SMC is critically involved in both, constitutive MMP-2 secretion and enhanced MMP-2 release, following ANG II stimulation. We postulate that an activated RAS alters the proteolytic balance of an atherosclerotic plaque via enhanced NAD(P)H-oxidase-dependent release of MMP-2 by SMC, which potentially contributes to plaque destabilization.

An activated RAS contributes to an acute coronary event [18] and its effector peptide ANG II is mainly involved in the pathogenesis of atherosclerosis [12]. Since MMPs contribute to the progression of atherosclerotic plaque formation [19] via enhanced extracellular matrix decomposition we assessed whether MMP-2 and p47phox are co-localized with components of the RAS in human atherosclerotic lesions. We could demonstrate a tied association of MMP-2 and p47phox with ANG II and AT_1-receptor in α-SMC actin-rich areas in atherosclerotic plaques suggesting a functional interaction of these proteins.

Maladaptive remodeling of the vessel wall due to atherosclerosis may involve the induction of MMPs. We therefore investigated whether MMP-2 is enhanced in atherosclerotic LAD coronary arteries from patients suffering from ICM. We could show enhanced MMP-2 protein expression and gelatinolytic activity in these specimens compared to LAD coronary arteries from patients with DCM who do not suffer from atherosclerosis. In contrast to murine SMC we were able to detect both, the pro- and the active form of MMP-2, in human atherosclerotic arteries. Therefore, it is tempting to speculate that in atherosclerosis other cells than SMC, e.g., inflammatory cells or endothelial cells, may be involved in the activation of pro MMP-2.

These results suggest that induction of MMP-2 contributes to vascular remodeling due to enhanced collagen deposition and extracellular matrix remodeling within the atherosclerotic lesion extending previous findings by Galis and colleagues who demonstrate that MMP-9 synthesis and activation is enhanced in human coronary artery plaques [1]. Taken together, these observations suggest that an activated RAS may contribute to atherosclerotic plaque remodeling and potential destabilization via a NAD(P)H-oxidase-dependent activation of MMP-2. These observations are consistent with the notion that the RAS plays an important role in both, the progression of atherosclerosis and the development of cardiovascular events, as indicated by experimental and clinical observations, e.g., in the HOPE trial [20,21,18].

Acknowledgments

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References

[1] Z.S. Galis, G.K. Sukhova, M.W. Lark, P. Libby, Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques, J. Clin. Invest. 94 (1994) 2493–2503.

[2] Z.S. Galis, M. Muszynski, G.K. Sukhova, E. Simon-Morrissey, E.N. Unemori, M.W. Lark, E. Amento, P. Libby, Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion, Circ. Res. 75 (1994) 181–189.

[3] P. Brenneisen, K. Briviba, M. Wlaschek, J. Wenk, K. Scharffetter-Kochanek, Hydrogen peroxide (H_2O_2) increases the steady-state mRNA levels of collagenase/MMP-1 in human dermal fibroblasts, Free Radic. Biol. Med. 22 (1997) 515–524.

[4] K. Grote, I. Flach, M. Luchtefeld, E. Akin, S.M. Holland, H. Drexler, B. Schieffer, Mechanical stretch enhances mRNA expression and proenzyme release of matrix metalloproteinase-2 (MMP-2) via NAD(P)H oxidase-derived reactive oxygen species, Circ. Res. 92 (2003) e80–86, Epub 2003 May 2015.

[5] S. Rajagopalan, X.P. Meng, S. Ramasamy, D.G. Harrison, Z.S. Galis, Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability, J. Clin. Invest. 98 (1996) 2572–2579.

[6] S.O. Yoon, S.J. Park, S.Y. Yoon, C.H. Yun, A.S. Chung, Sustained production of H(2)O(2) activates pro-matrix metalloproteinase-2 through receptor tyrosine kinases/phosphatidylinositol 3-kinase/NF-kappa B pathway, J. Biol. Chem. 277 (2002) 30271–30282, Epub 32002 Jun 30210.

[7] H.E. Van Wart, H. Birkedal-Hansen, The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family, Proc. Natl. Acad. Sci. USA 87 (1990) 5578–5582.

[8] N. Inoue, S. Takeshita, D. Gao, T. Ishida, S. Kawashima, H. Akita, R. Tawa, H. Sakurai, M. Yokoyama, Lysophosphatidylcholine increases the secretion of matrix metalloproteinase 2 through the activation of NADH/NADPH oxidase in cultured aortic endothelial cells, Atherosclerosis 155 (2001) 45–52.

[9] B. Schieffer, M. Luchtefeld, S. Braun, A. Hilfiker, D. Hilfiker-Kleiner, H. Drexler, Role of NAD(P)H oxidase in angiotensin II-induced JAK/STAT signaling and cytokine induction, Circ. Res. 87 (2000) 1195–1201.

[10] U. Landmesser, H. Cai, S. Dikalov, L. McCann, J. Hwang, H. Jo, S.M. Holland, D.G. Harrison, Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II, Hypertension 40 (2002) 511–515.

[11] M. Wang, G. Takagi, K. Asai, R.G. Resuello, F.F. Natividad, D.E. Vatner, S.F. Vatner, E.G. Lakatta, Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates, Hypertension 41 (2003) 1308–1316, Epub 2003 May 1312.

[12] K.M. Schmidt-Ott, S. Kagiyama, M.I. Phillips, The multiple actions of angiotensin II in atherosclerosis, Regul. Pept. 93 (2000) 65–77.

[13] B. Schieffer, E. Schieffer, D. Hilfiker-Kleiner, A. Hilfiker, P.T. Kovanen, M. Kaartinen, J. Nussberger, W. Harringer, H. Drexler, Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability, Circulation 101 (2000) 1372–1378.

[14] D. Goidin, A. Mamessier, M.J. Staquet, D. Schmitt, O. Berthier-Vergnes, Ribosomal 18S RNA prevails over glyceraldehyde-3-phosphate dehydrogenase and beta-actin genes as internal standard for quantitative comparison of mRNA levels in invasive and noninvasive human melanoma cell subpopulations, Anal. Biochem. 295 (2001) 17–21.

[15] G. Murphy, F. Willenbrock, T. Crabbe, M. O'Shea, R. Ward, S. Atkinson, J. O'Connell, A. Docherty, Regulation of matrix metalloproteinase activity, Ann. N. Y. Acad. Sci. 732 (1994) 31–41.

[16] F. Dol, G. Martin, B. Staels, A.M. Mares, C. Cazaubon, D. Nisato, J.P. Bidouard, P. Janiak, P. Schaeffer, J.M. Herbert, Angiotensin AT1 receptor antagonist irbesartan decreases lesion size, chemokine expression, and macrophage accumulation in apolipoprotein E-deficient mice, J. Cardiovasc. Pharmacol. 38 (2001) 395–405.

[17] M.R. Bergman, S. Cheng, N. Honbo, L. Piacentini, J.S. Karliner, D.H. Lovett, A functional activating protein 1 (AP-1) site regulates matrix metalloproteinase 2 (MMP-2) transcription by cardiac cells through interactions with JunB-Fra1 and JunB-FosB heterodimers, Biochem. J. 369 (2003) 485–496.

[18] S. Yusuf, P. Sleight, J. Pogue, J. Bosch, R. Davies, G. Dagenais, Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators, N. Engl. J. Med. 342 (2000) 145–153.

[19] H.R. Lijnen, Extracellular proteolysis in the development and progression of atherosclerosis, Biochem. Soc. Trans. 30 (2002) 163–167.

[20] F. Diet, R.E. Pratt, G.J. Berry, N. Momose, G.H. Gibbons, V.J. Dzau, Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease, Circulation 94 (1996) 2756–2767.

[21] S. Hoshida, J. Kato, M. Nishino, Y. Egami, T. Takeda, M. Kawabata, J. Tanouchi, Y. Yamada, T. Kamada, Increased angiotensin-converting enzyme activity in coronary artery specimens from patients with acute coronary syndrome, Circulation 103 (2001) 630–633.

[22] S.H. Jackson, J.I. Gallin, S.M. Holland, The p47phox mouse knock-out model of chronic granulomatous disease, J. Exp. Med. 182 (1995) 751–758.