Evidence for vascular macrophage migration inhibitory factor in destabilization of human atherosclerotic plaques

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Abstract

Objective: Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine and has been shown to play a role in pathogenesis of atherosclerosis. The aim of this study is to investigate the potential role of MIF in the destabilization of atherosclerotic plaques by stimulation of vascular MMP-1 expression.

Methods: MIF and matrix metalloproteinase protein-1 (MMP-1) expression in human atherosclerotic plaques were determined by immunohistochemistry. The functional activity of MIF was examined by its ability to induce MMP-1 expression in vascular smooth muscle cells (VSMCs) in vitro.

Results: Two-color immunohistochemistry demonstrated that MIF was strongly upregulated in vulnerable, but not in fibrous plaques. Upregulation of vascular MIF was associated with macrophage accumulation ($p<0.01$), strong expression of vascular MMP-1 ($p<0.001$), and collagenolysis in vulnerable atheromatous plaques, but not in the fibrous lesions. Co-expression of MIF and MMP-1 in vulnerable atheromatous plaques appeared to contribute to the weakening of fibrous caps and plaque disruption. The role of MIF in vascular MMP-1 expression was demonstrated by the ability of MIF to directly stimulate VSMCs to express MMP-1 mRNA and protein, and to increase MMP-1 activity in a dose- and time-dependent manner, which was blocked by a neutralizing MIF antibody ($p<0.001$).

Conclusions: MIF and MMP-1 are markedly upregulated in vulnerable atheromatous plaques and are associated with the weakening of the fibrous cap. The ability of MIF to induce MMP-1 expression and collagenolytic activity in VSMCs suggests that MIF may play a role in the destabilization of human atherosclerotic plaques.

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Keywords: Atherosclerosis; Cytokines (MIF); Matrix metalloproteinases; Macrophages; Smooth muscle cells

1. Introduction

Atherosclerosis is an inflammatory process characterized by the accumulation of lipid-rich macrophages, vascular smooth muscle cells (VSMCs), lipids, and extracellular matrix (ECM) [1–4]. Atherosclerosis complicated by plaque rupture or thrombosis is a major cause of potentially lethal acute coronary syndromes and stroke [2–4]. Rupture occurs frequently in plaques containing a soft, lipid-rich core that is covered by a thin and inflamed cap of fibrous tissue [3,4]. It has been demonstrated that the ruptured plaques usually have thinner caps, with less collagen, fewer VSMCs, and

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more macrophages [3,4]. Therefore, the major determinants of plaque vulnerability and rupture are progressive lipid accumulation, ongoing inflammation, and cap weakening, which are associated with increased collagen degradation and impaired healing and repair process by VSMCs [2–4]. It is generally accepted that matrix metalloproteinases (MMPs) play an essential role in plaque instability [5–7]. Of them, MMP-1 is believed to be important since it is responsible for the initial cleavage of fibrillar collagen type I and III, the major matrix components in atherosclerotic plaques [8,9]. MMP-1 is upregulated in human advanced atherosclerotic plaques and aneurysmal lesions [10]. In addition, MMP-1 is also highly expressed in rabbit atheromatous lesions induced by balloon injury andatherogenic diet, but was downregulated by cholestero- lowering drugs which are associated with stabilization of the atheromatous lesions [11]. Many inflammatory factors such as oxidized LDL [12], hypercholesterolemia [11], inflammatory cytokines such as IL-1β and TNF-α [12–14], and C-reactive protein [15], all have been shown to upregulate MMP-1 expression by endothelial cells and VSMCs.

Macrophage migration inhibitory factor (MIF) is a unique pro-inflammatory cytokine and is crucial in regulating immune-mediated diseases including septic shock [16,17], arthritis [18], acute respiratory distress syndrome [19], glomerulonephritis [20,21], and allograft rejection [22]. Recently, we and other investigators also demonstrated that MIF participates in both experimental and human atherosclerosis [23,24]. MIF is expressed by vascular endothelial cells, VSMCs, and macrophages [23,24]. Upregulation of vascular MIF is associated with macrophage adhesion, accumulation, and foam cell transformation during atherogenesis [23]. Blockade of MIF using a neutralizing MIF antibody reduces vascular inflammation, cell proliferation, and neointimal thickening [25]. Furthermore, deficiency of MIF attenuates atherogenesis in low-density lipoprotein receptor-deficient (LDLr−/−) mice [26]. Direct evidence for the role of MIF in destabilization of atheromatous plaques comes from a recent study in Apolipoprotein E-deficient mice [27]. In this study, inhibition of MIF with a neutralizing MIF antibody resulted in a shift in the cellular composition of neointimal plaques toward a stabilized phenotype with reduced macrophage/foam cell content and increased SMC content [27]. These observations underscore a potentially important role for MIF in atherosclerosis and identify a new potential target for modulating atherogenesis. In addition, MIF has been shown to stimulate MMP-1 and MMP-3 mRNA expression in synovial fibroblasts obtained from patients with rheumatoid arthritis and to upregulate MMP-9 and MMP-13 in rat osteoblasts [28,29]. These findings suggest a pathogenic role for MIF in the destruction of joint tissues and cartilage in arthritis. However, the precise mechanism whereby MIF induces the instability of atherosclerotic plaques has not been fully understood. We hypothesize that induction of MMP-1 expression by VSMCs may be a mechanism by which MIF induces the destabilization of atherosclerotic plaques.

2. Methods

2.1. Human arterial tissues

Surgical specimens of human atherosclerotic arteries were obtained from 42 patients (31 male, 11 female, mean age 62.3±7.2 years) undergoing femoral bypass (n=29), and repair of an abdominal aortic aneurysm (n=7). Surgical specimens of renal arterial atherosclerosis were obtained from patients undergoing unmatched kidney transplantation (n=6) at the First Foshan Hospital, Guangdong, China, and at the North Shore University Hospital, NY, USA. In addition, five normal renal arteries obtained from unmatched kidney transplantation donors were used as normal controls. The study protocols were approved by the Human Investigation Review Committee at both Institutions and conformed with the Declaration of Helsinki. Atherosclerotic plaques were classified as previously described [9]. Plaques with thickness of fibrous cap $\geq 0.8$ mm, fibroblasts/VSMCs and collagen matrix $\geq 10\%$, and macrophages and lipid content $\leq 10\%$ were designated as fibrous (Fig. 1F), whereas, lesions with fibrous cap thickness $\leq 0.3$ mm, fibroblasts/VSMCs and collagen matrix $\leq 10\%$, and macrophages and lipid content more than $\geq 20\%$ were designated as vulnerable plaques (Fig. 1B). If lesions were complicated by plaque rupture, they were classified as vulnerable plaques regardless of the cap thickness in the intact cap area (Fig. 1D). Of these, 16 cases (6 from patients with abdominal aneurysm, 6 from those with femoral bypass, and 4 from those with renal arterial atherosclerosis) were classified as vulnerable atheromatous plaques including two cases with plaque rupture and 18 cases (all from patients with femoral bypass) as fibrous plaques. Eight cases (one from a patient with abdominal aneurysm, two from renal artery, and five from those with femoral bypass) were between fibrous and vulnerable plaques and were excluded from this study.

2.2. Antibodies and recombinant human MIF

The mouse anti-MIF monoclonal antibody (mAb) [22,23] and rabbit anti-human MMP-1 antibody (Chemicon, Temecula, CA) were used in this study. In addition, polyclonal rabbit antibodies that recognize human collagen type I collagen (Santa Cruz Biotech., Santa Cruz, CA) or is reactive to the carboxy-terminal COL2-3/4C short neoepitope generated by cleavage of native human collagen by human collagenase MMP-1 (C1, 2C, IBEX Diagnostics, Montreal, Quebec, Canada) were also used. Other antibodies used in this study include: KP-1, a mouse anti-human CD68 mAb (that recognizes monocytes/macrophages); UCHL1, a mouse anti-CD45RO mAb that recognizes mature, activated...
T-cells and a subset of resting T-cells; a mouse anti-human α-smooth muscle actin mAb; a rabbit anti-human GAPDH; goat anti-mouse IgG; swine anti-rabbit IgG; mouse or rabbit peroxidase anti-peroxidase complexes (PAP); mouse alkaline phosphatase anti-alkaline phosphatase complexes (APAAP), and DAKO Enversion+Peroxidase System (rabbit). All antibodies were purchased from Dako (Dako, Capinteria, CA). Recombinant human MIF (rhMIF) was cloned, expressed in *Escherichia coli* and purified from the soluble fraction of the cell lysate by two-step high-pressure liquid chromatography (HPLC) as follows: (i) size exclusion HPLC on Bio-Sil TSK 250 (Bio-Rad, Munich, Germany) and (ii) ion-exchange HPLC on Ultrasphere TSK CM-3SW (LKB/Pharmacia, Freiburg, Germany) as described previously [30]. rhMIF contained <10 pg of endotoxin per microgram of recombinant protein as determined by the chromogenic *Limulus* amoebocyte assay (Chromogenix, Mölndal, Sweden).

2.3. Immunohistochemistry

Collected arteries were cut and serial sections (4 μm) were stained with one- and two-color immunohistochemistry using a previously described microwave-based method [20–23,31]. Briefly, after microwaving, sections were labeled with KP1, UCHL1, MMP-1, or α-SMA antibodies using a three-layer PAP, and developed with 3,3-diaminobenzidine to produce a brown product. For double immunostaining, a second round of microwave oven heating was used to denature bound immunoglobulins within the tissue, thereby preventing antibody cross-reactivity [28]. Sections then were labeled with the anti-MIF mAb or anti-MMP-1 mAb using a three-layer APAAP method, and developed with Fast Blue BB Salt (Ajax Chemicals, Melbourne, Australia). Sections were mounted in an aqueous medium and examined under microscopy.

Fig. 1. Double-immunohistochemistry demonstrates that MIF and MMP-1 expression by VSMCs in vulnerable, but not in fibrous plaques. MIF and MMP-1 are labeled as blue and VSMCs labeled with the anti-α-SMA Ab are brown. Note that co-expression of MIF (or MMP-1) and α-SMA is labeled as purple. (A, B) A normal human artery. MIF (A) and MMP-1 (B) are weakly expressed by VSMCs. (C, D) A set of serial sections of a vulnerable atheromatous plaque from a patient with abdominal aneurysmal repair. MIF expression (C) is markedly upregulated and is associated with strong expression of MMP-1 (D) by VSMCs, leading to weakening of fibrous cap. (E, F) A set of serial sections of fibrous plaque from a patient with femoral bypass. Note that fibrous cap is thickened and MIF (E) and MMP-1 (F) are not upregulated by VSMCs. *Lipid-core lesions. Magnifications: ×200.
A mouse anti-rat CD45 mAb (OX-1) and normal rabbit IgG, which do not react with human tissues, were used as negative controls.

2.4. Cell culture

Characterized rat aorta-derived smooth muscle cells were kindly provided by Dr. Andrew Kahn (University of Texas, Houston, TX, USA) [32]. Cells (passage 8) were cultured in DMEM containing 10% fetal bovine serum (FBS) until sub-confluence. Cells then were serum starved for 24 h and recombinant human MIF (rhMIF) at doses of 0, 10, 25, 50, 100 ng/ml were added into the culture in the presence or absence of a neutralizing MIF mAb (IgG1, 10–20 μg/ml) or an isotype control mAb (73.5 that specifically recognizes the human CD45RO antigen, IgG1, 10–20 μg/ml) for 0, 3, 6, 12, and 24 h. MMP-1 mRNA was examined by real-time PCR and protein expression by Western blot analysis.

2.5. MMP-1 mRNA detection by real-time PCR

RNA isolation for real-time PCR was performed as previously described [23]. Real-time, one step RT-PCR was performed with SYBR Green PCR Reagents (Sigma), the Thermoscript RT-PCR system (Invitrogen) and the Opticon DNA Engine (MJ Research), according to manufacturer’s instructions; 100 ng of total RNA was reversibly transcribed prior to PCR as follows: 94°C for 2 min followed by 40 cycles of denaturation, annealing, and extension at 94°C for 15 s, 58°C for 30 s, 72°C for 30 s each, respectively, and final extension at 72°C for 10 min. Primers used for detection of MMP-1 mRNA were: forward 5'-ATGTG-GATGCTGCATAAGAGC-3' and reverse, 5'-GACAG-CATCTACTTTGGTCCGC-3'. PCR reaction for each sample was done in triplicate for both target genes and for the GAPDH control. Ratios for MMP-1/GAPDH mRNA were calculated for each sample and expressed as the mean±S.D. At least three independent experiments were performed.

2.6. MMP-1 protein detection by Western blotting

As described previously [9], 20 μg of protein extracts were mixed with SDS-PAGE sample buffer, boiled for 5 min, electrophoresed on a 10% SDS polyacrylamide gel, and electroblotted onto Hybond-ECL nitrocellulose membrane (Amersham International, Buckinghamshire, UK). After blocking, the membrane was incubated with rabbit antibodies to MMP-1, followed by the secondary anti-rabbit antibody (1:20,000) and developed using the ECL detection kit (Amersham) to produce a chemiluminescent signal. Densitometric analysis was performed with NIH Image software. Ratios for MMP-1/GAPDH protein were calculated for each sample and expressed as the mean±S.D. At least three independent experiments were performed.

2.7. Analysis of MMP-1 activities

Two methods were applied to detect MMP-1 activity. Firstly, in situ collagenase-cleaved interstitial type I collagen in atherosclerotic plaque lesions was detected by immunostaining with a polyclonal rabbit antibody reactive to cleavage of native human collagen by human collagenase MMP-1 (C1, 2C, IBEX Diagnostics). The antibody has been shown to specifically detect collagenase-cleaved type I collagen in human vulnerable atheromatous plaques without immunoreactivity to native or denatured human type I or III collagens [9]. Briefly, after microwaving, paraffin-sections were incubated overnight with either rabbit anti-human collagen I (Santa Cruz) or COL2-3/4Cshort (C1, 2C) antibodies, followed by peroxidase-conjugated goat anti-rabbit antibody and then DAKO Envision+ Peroxidase (rabbit), and developed with DAB to produce brown products. Secondly, MMP-1 activity in cultured medium under various conditions was measured by ELISA in triplicates using The CHEMICON MMP Collagenase Activity Assay Kit (Chemicon International), according to the manufacturer’s protocol. MMP-1 collagenase activity was determined using biotinylated bovine native collagen as substrate. The cleaved biotinylated fragments of collagen by MMP-1 were transferred to biotin-binding 96-well micro-titer plates and detected by streptavidin-peroxidase complex and enzyme substrate. The optical density of the wells was determined. Collagenase activity was calculated against standards with pre-activated human MMP-1 and data was expressed as the mean±S.D.

2.8. Quantitation of immunohistochemistry

The number of KP-1, UCHL1 positive cells in atherosclerotic plaques was counted by means of a 0.02 mm² graticule fitted in the eyepiece of the microscope, and expressed as cells per mm². Since MIF and MMP-1 were widely expressed in both vulnerable and fibrous plaques, their expressions were determined by quantitative Image Analysis System (Optima 6.5, Media Cybernatics, Silver Springs, MD). Briefly, the examined area of fibrous caps and shoulders was outlined and the positive staining patterns were identified, then the percent positive area in the examined area was measured. The lipid and necrotic core, and vascular lumens were excluded from the study. All scoring was performed blinded on coded slides and data expressed as the mean±S.D.

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism3.0 (GraphPad Software, San Diego, CA, USA). Differences in MIF and MMP-1 expression, macrophage and T cell accumulation between vulnerable and fibrous plaques were assessed by unpaired t-test, while correlation between MIF expression and macrophage or T cell...
accumulation, and MMP-1 expression was analyzed by Pearson linear correlation analysis.

3. Results

3.1. MIF and MMP-1 expression by vascular smooth muscle cells in human vulnerable and fibrous plaques

As shown in Fig. 1 A and B, two-color immunohistochemistry demonstrated that MIF and MMP-1 were weakly expressed by α-SMA+VSMCs in normal human arteries. However, marked upregulation of MIF and MMP-1 was found in vulnerable atheromatous plaques as demonstrated by a large number of MIF+α-SMA+ (Fig. 1C) or MMP-1+α-SMA+ (Fig. 1D) cells in the fibrous cap, leading to weakening of the cap. In contrast, expression of MIF and MMP-1 by VSMCs was largely reduced in fibrous plaques with thickening of fibrous caps (Fig. 1E, F). Interestingly, α-SMA+ cells with strong expression of MIF and MMP-1 were enlarged in size in the vulnerable cap (Fig. 1C, D), while those with weak MIF and MMP-1 expression showed elongated, dense, and small size in the thickened fibrous cap (Fig. 1E, F). These changes may be associated with their functional activities.

3.2. MIF expression and macrophage and T cell accumulation in human vulnerable and fibrous plaques

Two-color immunohistochemistry also showed that MIF was strongly expressed within vulnerable atheromatous plaques by CD68+ macrophages (Fig. 2B). In

![Fig. 2. Double-immunohistochemistry demonstrates that MIF and MMP-1 are strongly co-expressed in vulnerable, but not in fibrous plaques. (A) A normal human artery. MIF (blue) and MMP-1 (brown) are weakly expressed by vascular endothelial cells and VSMCs. (B and C) A set of serial sections of a vulnerable atheromatous plaque from a patient with femoral bypass. MIF expression (blue) is markedly upregulated and is associated with numerous CD68+ macrophage (brown) accumulation and weakening of the fibrous cap (B), which is associated with marked upregulation of MMP-1 (brown, C). Note that the secreted MIF (blue) is evident in the lipid-core lesion (B). (D) A ruptured plaque from a patient with abdominal aortic aneurysm. Note that MIF (blue) and MMP-1 (brown) are co-expressed and markedly upregulated by morphologically elongated cells (presumably VSMCs and myofibroblasts, see insert picture) in the fibrous cap and shoulder with plaque rupture (arrow). (E) A fibrous plaque from a patient with femoral bypass. Note that fibrous cap is thickened and MIF (blue) is not upregulated with a few CD68+ macrophages (arrowheads). (F) A fibrous plaque from a patient with femoral bypass. Note that both MIF (blue) and MMP-1 (brown) are not upregulated in a fibrous plaque. *Lipid-core lesions. Magnifications: ×100.](https://academic.oup.com/cardiovascres/article-abstract/65/1/272/309981)
addition, secreted MIF was also found within the plaque, particularly in the core-lesion (Fig. 2B). Strikingly, upregulation of MIF in vulnerable plaques was associated with the numerous macrophage accumulation (Figs. 2B and 3). This was profound in the inflamed shoulder and cap and was associated with weakening of fibrous caps (Fig. 2B). In contrast, stable fibrous plaques had thickened fibrous cap and a few macrophages without significant upregulation of MIF (Figs. 2E and 3). Only a few T cells (UCHL1+) within the atheromatous plaques were noted. Semi-quantitation of macrophages and T cells in both vulnerable and fibrous plaques is shown in Fig. 3B.

3.3. Co-expression of MIF and MMP-1 in human vulnerable and fibrous plaques

MMP-1 was weakly expressed and co-localized with MIF expression in both VECs and VSMCs in normal human arteries (Fig. 2A). However, two-color immuno-histochemistry showed that MMP-1 was markedly upregulated and co-localized with MIF expression in vulnerable atheromatous plaques (Fig. 2C). As indicated in a set of serial sections, CD68+ macrophages and morphologically elongated VSMCs or myofibroblasts were the major cell types for dual expression of MIF and MMP-1 (Fig. 2B vs. C). Importantly, marked upregulation of MIF and MMP-1 in vulnerable plaques was associated with weakening of the fibrous cap (Fig. 2C). Notably, in two cases with progressive plaque disruption, elongated VSMCs and myofibroblasts were a major source of both MIF and MMP-1 in the ruptured fibrous cap (Fig. 2D). In contrast, there was no significant MIF and MMP-1 expression in fibrous plaques and fibrous cap (Fig. 2E, F). Semi-quantitation of MIF and MMP-1 expression in both vulnerable and fibrous plaques was shown in Fig. 3A.

3.4. Upregulation of MIF and MMP-1 is associated with an increase in collagenolysis in vulnerable atheromatous plaques

Since upregulation of immunoreactive MMP-1 in vulnerable atheromatous plaques does not necessarily
reflect the activity of MMP-1, we examined MMP-1 collagenase activity by immunostaining for the collagenase-cleaved type I collagen fibrils in sets of serial sections. As shown in Fig. 4, immunohistochemistry revealed that there was a marked increase in immunoreactive type I collagen (Fig. 4A, C) and reduction in intact type I collagen accumulation (Fig. 4B, D) in the cap and shoulder of vulnerable or ruptured plaques. An increase in collagenolysis in vulnerable and ruptured plaques was associated with upregulation of MIF and MMP-1 as demonstrated in serial sections (Figs. 2B–D). In contrast, fibrous plaques showed little cleavage of type I collagen with abundant intact type I collagen accumulation in the thick fibrous cap (Fig. 4E, F). This was also associated with reduction of MIF and MMP-1 expression in serial sections (Fig. 2E, F).

3.5. Correlation of MIF expression and macrophages, T cells, and MMP-1 expression in human atherosclerotic plaques

As shown in Fig. 5, correlation analysis revealed that upregulation of MIF in both vulnerable and fibrous plaques was significantly correlated with macrophage, but not T cell, accumulation (Fig. 5A, B). Further analysis also showed that upregulation of MIF was highly correlated with an increase in MMP-1 expression (Fig. 5C).

3.6. MIF is able to induce MMP-1 expression and activity by VSMCs in vitro

Since VSMCs and macrophages were the major cell types producing MIF (Figs. 1B and 2B) during the development of atherosclerotic plaques and because expression of MMP-1 by VSMCs was tightly associated with the weakening of fibrous cap or plaque rupture (Figs.
we postulated that VSMCs and macrophages-derived MIF may be responsible for the upregulation of MMP-1 by VSMCs in vulnerable plaques. Since VSMCs are the major cell type with strong MIF and MMP-1 expression in fibrous caps of vulnerable plaques as shown in Fig. 1C and D and because the functional activities of VSMCs in the cap are a critical determinant of plaque stability [2–4], the functional role of MIF in induction of MMP-1 expression and activity was investigated by stimulating VSMCs with MIF in vitro. As shown in Fig. 6, although real-time PCR demonstrated that constitutive expression of MMP-1 mRNA was low in normal VSMCs (Fig. 6A), addition of MIF strongly induced MMP-1 mRNA expression by VSMCs in a time- and a dose-dependent manner (Fig. 6A, B), being significant at 3 h (Fig. 6A). The specificity of MIF to induce MMP-1 mRNA expression in VSMCs was further demonstrated by the addition of a neutralizing MIF mAb. As shown in Fig. 6B, MIF-induced MMP-1 mRNA expression by VSMCs at 3 h was substantially blocked by a neutralizing MIF mAb, but not by an isotype control Ab.

Next, we further analyzed the ability of MIF to induce MMP-1 protein expression by VSMCs. As shown in Fig. 7, addition of MIF induced MMP-1 protein expression in a time- and a dose-dependent fashion (Fig. 7A, B). Again, addition of a neutralizing MIF antibody, but not an isotype control antibody, was able to block MIF-induced MMP-1 protein synthesis by VSMCs (Fig. 7C), indicating the specificity of MIF to induce MMP-1 protein expression.

MIF-induced MMP-1 activity measured by The CHEMICON MMP Collagenase Activity Assay Kit was shown in Fig. 8. Indeed, MIF was able to significantly increase MMP-1 collagenolytic activity in a dose-dependent manner, which

![Graph showing time-dependent and dose-dependent MMP-1 expression](image-url)
compared to MIF (50 ng/ml) and CTL Ab.

...arteriosclerotic plaques[27]. In the present study, we extend...MIF with a neutralizing MIF antibody to stabilize atherosclerotic plaques in apolipoprotein E-deficient mice indi-

...sclerosis-susceptible mice in which vascular inflammation and neointimal thickening after angioplasty are...of atherosclerosis has been further supported by a study finding that LDLr/C0/C0 mice null for MIF have impaired...arteriosclerotic plaques [23]. The potential importance of MIF in atherosclerosis is confirmed by the...in vulnerable atheromatous plaques by stimulating vascular MMP-1 expression and activity in vulnerable human atheromatous plaques.

...is well documented that the integrity of the fibrous cap is important for the stability of atherosclerotic plaques, which depends largely on the collagenous extracellular matrix produced by VSMCs or myofibroblasts [3,4]. An increased inflammatory response, decreased ECM synthesis, and increased ECM degradation contribute significantly to plaque instability [1–4]. Increased MMP activity appears to be the most important risk factor for the instability of vulnerable atheromatous plaques or plaque rupture [2–8]. Several MMPs including MMP-1, -2, -3, -9, -13, and -14 may be involved [2–8]. Among them, MMP-1 has been considered as a highly specific protease capable of degrad-

...expression and activity in vulnerable human atheromatous plaques. Because of the functional specificity of MMP-1 and its localization in human vulnerable atheromatous plaques with active collagenolysis [9,33], it has been hypothesized that MMP-1 contributes to the expansion and rupture of the plaque. In the present study, the finding that upregulation of MIF and MMP-1 is associated with marked collagenolysis in vulnerable plaques suggest a causal role of MIF in the destabilization of the plaque. Although macrophages and VSMCs/myofibroblasts are two major cell types expressing MMP-1, the functional activity of this enzyme in atherogenesis and plaque complications may be cell-type specific. Transgenic overexpression of MMP-1 in macrophages has been noted to produce protective effects on the atherogenesis with smaller and less advanced lesions in ApoE knockout mice [34]. This may be associated with the findings that the degradation of type I collagen by MMP-1 prevents the differentiation of monocytes into macrophages and impedes VSMC migration to the lesion, the critical process in atherogenesis [34]. In addition, since oxidized LDL binds collagen types I and III efficiently [35], expression of MMP-1 on macrophages may degrade LDL-bound collagens, thereby reducing neointimal retention of oxidized LDL [34]. Thus, transgenic over-expression of MMP-1 in macrophages delays atherogenesis. In contrast, as shown in the present study, once the atherosclerotic plaque is formed, expression of MMP-1 by VSMCs and myofibroblasts, as well as macrophages, in vulnerable plaques degrade the collagen matrix and impair...
the healing process, resulting in the weakening of fibrous cap and plaque rupture. Therefore, upregulation of MMP-1 in vulnerable atheromatous plaques may play a critical role in plaque expansion and rupture.

Several inflammatory mediators such as IL-1, TNF-α, and OxLDL have been shown to increase the expression of MMP-1 by VSMCs, endothelial cells, and macrophages [8–14]. The present study provides the first evidence that MIF, which is considered to be a high, upstream activator of the pro-inflammatory response, also contributes to the pathophysiology of plaque instability by activating MMP-1. Indeed, MIF is constitutively expressed by many cells including macrophages, endothelial cells, and VSMCs [23,24] and it is released immediately from pre-formed pools upon stimulation with endotoxin, exotoxin, TNF-α released immediately from pre-formed pools upon stimulation with endotoxin, exotoxin, TNF-α, interferon-γ, and OxLDL [16–18,21]. Once released, MIF acts by autocrine and paracrine pathways to further stimulate the expression of cytokines (IL-1, TNF-α, INF-γ) and adhesion molecules [16–21]. Thus, it is likely that the local production of vascular MIF induced by OxLDL not only causes macrophage adhesion, migration, and foam cell transformation during atherogenesis [23,24], but also contributes to the instability of the plaques by stimulating MMP-1 expression by VSMCs directly, and indirectly by the induction of other inflammatory cytokine cascades.

In summary, this study demonstrates that MIF and MMP-1 are strongly co-expressed in vulnerable human atheromatous plaques and may be associated with the instability or rupture of atherosclerotic plaques. The ability of MIF to activate vascular MMP-1 and may be a key mechanism by which MIF mediates the destabilization of the atherosclerotic plaques.

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