Decreased fibrocyte number is associated with atherosclerotic plaque instability in man

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Aims
Plaque rupture partly results from inadequate collagen synthesis due to lower smooth muscle cell numbers in fibrous caps. Fibrocytes are bone-marrow-derived circulating mesenchymal progenitors and have recently been identified in fibrous caps. This study hypothesized that reduced fibrocyte numbers would be associated with plaque instability.

Methods and results
Patients with acute myocardial infarction (MI) (n = 22), stable angina (SA) (n = 20), or healthy controls (n = 22) were recruited. Circulating fibrocytes (CD45⁺/CD34⁺/collagen I⁺) were measured by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated from blood and cultured for 2 weeks, and fibrocytes were quantified by morphology (spindle-shaped) and flow cytometry (CD45⁺/collagen I⁺). Another set of PBMCs was stimulated with macrophage colony-stimulating factor (M-CSF) for 72 h and the expression of several macrophage markers was measured by flow cytometry. Acute MI patients had decreased circulating fibrocyte numbers compared with healthy controls or SA patients. Following 2 weeks’ culture, both the number of spindle-shaped fibrocytes counted under the microscope and the percentage of fibrocytes of the remaining adherent cells in culture measured by flow cytometry were reduced in acute MI patients. Expression of macrophage markers CD68, CD36, and EMR in M-CSF-stimulated PBMCs was enhanced in acute MI patients compared with the other two groups. SA patients with previous MI had decreased circulating fibrocyte numbers and a lower yield of fibrocytes from PBMCs than those without previous MI.

Conclusions
This is the first report of decreased fibrocyte numbers in patients with MI. Reduced fibrocytes and preferential differentiation of PBMCs into macrophages may contribute to plaque instability.

Keywords
Plaque instability • Fibrocytes • Macrophages • Monocytes • Myocardial infarction

1. Introduction
The rupture of an atherosclerotic plaque, which leads to myocardial infarction (MI), stroke, and death, is a critical clinical issue. There are currently no ideal methods that can prevent or predict plaque rupture. An unstable plaque usually has a thin fibrous cap and a large necrotic lipid core.¹ The ruptured fibrous cap is known to be rich in macrophages that produce matrix metalloproteinases (MMPs), thus digesting extracellular matrix (ECM) and weakening the fibrous cap, but is usually poor in smooth muscle cells, the primary cells responsible for the synthesis of ECM.² So, both enhanced proteolytic activity due to an increase in the number of macrophages and insufficient fibrous cap repair due to a decrease in the number of smooth muscle cells contribute to plaque instability and vulnerability.³

A new population of matrix-secreting cells, termed fibrocytes, was first reported in 1994.⁴ These cells are bone marrow-derived mesenchymal progenitors in circulation. Initial studies by Bucala et al.⁴ demonstrated that peripheral blood mononuclear cells (PBMCs) were capable of developing into fibrocytes (appearing as spindle-shaped cells) after 10–14 days of culture. Later studies further identified that fibrocytes were derived specifically from the precursors of the monocytic lineage and in particular CD14⁺ monocytes.⁴–⁶ Fibrocytes express a variety of mesenchymal markers including collagen I and vimentin, the leucocyte common antigen CD45, and the haematopoietic stem cell marker CD34,⁷ which is down-regulated with
recruitment of the cells into tissue. They produce matrix proteins such as collagen and vimentin and migrate to the sites of tissue injury where they differentiate into fibroblast-like cells. Fibrocyte differentiation is promoted by transforming growth factor-β (TGF-β) and Th-2 cytokines, such as interleukin (IL)-4 and -13, and inhibited by Th-1 cytokines such as interferon-γ (IFN-γ), and IL-12. Serum amyloid P (SAP) is another potent inhibitor of fibrocyte differentiation, while cytokine leukotrienes (cysLTs) are reported to induce fibrocytes proliferation in an autocrine and paracrine manner. Migration of fibrocytes are mainly mediated by the interactions between chemokines [stromal cell-derived factor (SDF)-1, secondary lymphoid-tissue chemokine (SLC)] and their respective receptors expressed on fibrocytes (CXCR4 and CCR7). A growing body of evidence suggests that fibrocytes not only participate in normal wound healing and tissue regeneration, but are also involved in aberrant wound healing and various fibrotic disorders. Several studies further demonstrate that lesional fibroblasts mature at least in part from fibrocytes.

Fibrocytes co-expressing pro-collagen I and CD34 have been recently identified in the fibrous cap of human atherosclerotic lesions, implying that fibrocytes may contribute to the formation of the fibrous cap. Given the ability of fibrocytes to secrete matrix proteins, we hypothesize that decreased fibrocytes might be associated with plaque rupture. Furthermore, since both fibrocytes and macrophages originate from bone marrow-derived precursors of the monocyte lineage, the preferential differentiation of these precursors into macrophages rather than fibrocytes may exist in MI patients further contributing to plaque instability.

2. Methods

2.1 Study subjects

We studied 22 patients with acute MI presenting with chest pain ≥ 30 min in duration, confirmation of infarct-related artery disease on coronary angiography. All patients underwent primary percutaneous coronary intervention. Aspirin, heparin, and nitrates were given to all patients. The use of β-blockers, calcium channel blockers, angiotensin-converting enzyme-inhibitors, and nitrates was at the discretion of the attending physician. Twenty-two patients with chronic stable angina (SA) and 22 healthy volunteers were recruited. Patients with SA had no evidence of recent deterioration or rest pain in the previous 6 months. Medical records of all patients with SA were examined to determine whether or not they had a known admission with MI [ST elevation MI (STEMI) or non-ST elevation (NSTEMI)]. The healthy volunteers had no evidence of cardiovascular disease as determined by history, physical examination, and ECG. Exclusion criteria included current or recent use of immunosuppressive agents, chronic infection such as HIV or hepatitis, known pulmonary hypertension, renal or neoplastic disease, and any surgical procedures in the preceding 6 months. The sample size was determined according to a prior power analysis (power of 0.9). This study complied with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Alfred Healthcare. Informed consent was obtained from all participants.

2.2 Blood sample collection

Blood samples (30 mL) were collected into EDTA tubes by venipuncture from patients with acute MI immediately on admission (196 ± 74 min from symptoms to sampling, before the interventional procedure), SA patients and healthy controls. 2–3 mL blood was sent to Alfred Pathology Services for full blood count measurement. A further 2 mL of whole blood was allocated for measuring circulating fibrocytes by flow cytometry (see below). Plasma samples (10 min centrifugation at 400 g followed by a further 10 min at 600 g) were stored at −80°C for cytokine and chemokine measurement (detailed below). The remaining blood (after removal of plasma) was diluted with phosphate-buffered saline (PBS) and used for PBMC isolation as per below.

2.3 Measurement of circulating fibrocytes by flow cytometry

200 μL of whole blood was aliquoted to each tube for flow cytometry. After red blood cells were lysed, leucocytes were incubated with anti-collagen I antibody (Millipore) for 30 min on ice, and washed twice with PBS containing 1% foetal bovine serum (FBS), followed by incubation with secondary anti-mouse antibody conjugated to FITC (Millipore) for 30 min on ice and washed twice. Cells were then further stained with APC-conjugated anti-CD45 antibody and PE-conjugated anti-CD34 antibody or respective isotype control antibodies (BD Bioscience) for 30 min on ice and washed twice, and then fixed in 2% formaldehyde solution. Leucocytes (~500 000/sample) were acquired with a Becton-Dickinson FACs Calibur flow cytometer and data analysed with FlowJo software (Tree Star). Compensation was calculated with CompBeads (BD Bioscience) stained with single-colour antibody (FITC, PE, and APC). CD45+ cells were gated using isotype control and cells gated for CD45 were further analysed for collagen I and CD34 expression using isotype controls.

2.4 Measurement of fibrocytes from PBMC culture

The method of culturing fibrocytes from PBMCs was modified according to Quan and Bucala. PBMCs were isolated with the use of Ficoll-Paque plus (Amersham Biosciences) according to the manufacturer’s instruction. As described above, remaining blood was diluted with PBS (1:2) and layered on the top of Ficoll-Paque and centrifuged at 400 g for 30 min at room temperature. The mononuclear cell layer was carefully collected and rinsed twice with PBS. PBMCs were suspended in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 1% antibiotics-antimycotics, and plated at a density of 2 × 10⁶ cells/mL in fibronectin-coated 6-well plates (2.5 mL/well) and incubated at 37°C with 5% CO₂. After 3 days, non-adherent cells were removed and adherent cells were further cultivated until 2 weeks. Fibrocytes were measured by two methods. Firstly, fibrocytes were counted in five random fields per well under ×40, using the criteria of adherent cells with an elongated spindle-shape and the presence of an oval nucleus. Secondly, fibrocytes were detached with accutase (Sigma) and aliquoted to 3 × 10⁶ cells per tube for flow cytometry in 100 μL PBS and then measured by flow cytometry using two markers CD45 and collagen I (since cultured cells gradually lost CD34 expression) as described above. About 2 × 10⁶ events were acquired for each sample.

2.5 Gene expression of matrix proteins and chemokine receptors on cultured cells by real-time PCR

Total RNA was isolated from adherent cells after 2-weeks’ culture with Trizol Reagent® (Invitrogen) following the manufacturer’s instructions. After DNase (Promega) treatment, RNA was reverse transcribed into first strand cDNA with the use of random primers and M-MLV reverse transcriptase (Invitrogen). Real-time quantitative PCR was performed using a SYBR green kit (Roche). Detection and analysis were performed on an ABI Prism 7500 system (Applied Biosystems). The transcript abundance was expressed as fold increase over the value of the healthy control group calculated by the 2−ΔΔCt method. The expression of targeted genes (collagen I, fibronectin, vimentin, CXCR4, and CCR7) was normalized to 18s (primer sequences in Supplementary material online, Table).
2.6 Plasma levels of cytokines and chemokines by multiplex assay

Plasma samples were collected and stored at −80°C until use. Plasma levels of cytokines/chemokines were measured using multiplex kits purchased from Millipore (human cytokine/chemokine Panel I (seven analytes: IL-4, IL-13, IFN-γ, IL-12, monocyte chemoattractant protein-1 (MCP-1), CD40 ligand (CD40L), and interferon gamma-induced protein 10 (IP-10)) and Panel II (two analytes: SDF-1, SLR), TGF-β1 single plex, and human neuro-degenerative disease Panel II (one analyte: SAP) according to the manufacturer’s instruction. The appropriate cytokine standards, plasma samples (25 µL), and fluorescent conjugated, antibody-immobilized beads were added to wells of a pre-wetted filtered plate and then were incubated overnight at 4°C. The following day, the plate was washed twice with wash buffer and then incubated with secondary detection antibody for 1 h, followed by subsequent incubation with strepavidin-PE for 30 min. After the plate was washed twice again with wash buffer, it was run on the luminex system (Biorad) with the addition of sheath fluid. Concentrations of different analytes in the plasma samples were determined by using respective standard curves generated in the multiplex assays. Neat plasma samples were used for all assays except for SAP and TGF-β1 (1:2000 and 1:30 dilution, respectively, using assay buffer provided in the kits).

2.7 Measurement of leukotrienes

LTC4, LTE4, and cysLTs were measured using commercial enzyme immunoassay (EIA) kits (Cayman Chemical Company). Briefly, LT standards (C4, E4, or cysteinyl), plasma (50 µL), LT AChE tracer and anti-LT monoclonal antibody were added into a 96-well plate pre-coated with mouse anti-rabbit IgG. The plate was then incubated for 18 h at 4°C. After incubation, the plates were washed five times in wash buffer, and 200 µL of Ellman’s reagent was added to each well. The plate was developed in the dark after 30–60 min at room temperature with shaking. The developed plate was read at a wavelength of 412 nm on a microplate reader (Biorad). LT concentrations were determined by comparing the absorbance of plasma samples to the LT standards using a four-parameter logistic curve fit. The EIA for cysLTs is reported to have specificity for LTC4, 100%; LTD4, 100%; and LTE4, 79%.

2.8 Differentiation of macrophages

PBMCs from a subset of subjects were cultured with DMEM supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 1% antibiotics-antimycotics in the presence of a macrophage colony-stimulating factor (M-CSF) and M-CSF, 50 ng/mL or vehicle for 72 h. Several macrophage markers were measured by flow cytometry as described above. The adherent cells were harvested by incubation with accutase (Sigma), aliquoted to 3 × 10^4 cells per tube for flow cytometry in 100 µL PBS, and then stained with either APC-conjugated clonal antibody were added into a 96-well plate pre-coated with mouse anti-rabbit IgG. The plate was then incubated with secondary detection antibody for 1 h, followed by subsequent incubation with strepavidin-PE for 30 min. After the plate was washed twice again with wash buffer, it was run on the luminex system (Biorad) with the addition of sheath fluid. Concentrations of different analytes in the plasma samples were determined by using respective standard curves generated in the multiplex assays. Neat plasma samples were used for all assays except for SAP and TGF-β1 (1:2000 and 1:30 dilution, respectively, using assay buffer provided in the kits).

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2.9 Statistics

Data were expressed as mean ± SEM unless otherwise stated. Graphpad Prism 5.0 was used for statistical analysis. The normal distribution of data was tested by the Kolmogorov–Smirnov test. To compare differences among the three groups, one-way ANOVA followed by the Newman–Keuls multiple comparison test was used for parametric data, while the Kruskal–Wallis test followed by Dunn’s multiple comparison test was used for non-parametric data. Student’s t-test or Mann–Whitney test was used for the subgroup comparison of the SA patients depending on whether or not data were normally distributed. The Chi-square test was used to compare discrete variables among groups. A difference of P < 0.05 (two-sided) was considered statistically significant.

3. Results

3.1 Baseline characteristics of study subjects

The baseline characteristics of the study participants are summarized in Table 1. Most of the participants recruited in this study were males. The mean age of the SA patient group was significantly older than the healthy control group. More patients with acute MI were smokers while no significant difference in the prevalence of other known risk factors, including body mass index, diabetes, hypertension, hyperlipidaemia or family history of coronary artery disease was observed between the acute MI and SA groups. White blood cell (WBC) count was higher in the acute MI patients compared with the other two groups, and monocyte count was higher in acute MI patients compared with healthy controls. However, there was no difference in % of monocytes in WBC among the three groups. Information on ST-segment elevation, MI location, and enzyme profiles in acute MI patients are also presented in Table 1.

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<th>Table 1 Basic demographics of study subjects</th>
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MI, myocardial infarction; CAD, coronary artery disease; WBC, white blood cells; STEMI, ST elevation MI; NSTEMI, non-ST elevation MI. Data are presented as mean ± SEM or percentage.

*P < 0.05 vs. controls.

†P < 0.05 vs. stable angina.
fresh blood by flow cytometry using three markers (CD45/CD34/colla-
gen I) (Figure 1A). The percentage of circulating fibrocytes was then
multiplied by WBC count to determine the concentrations of fibro-
cytes per microlitre of blood. Acute MI patients had both a lower per-
centage of fibrocytes relative to WBC (CD45+) (P < 0.01) and a
lower concentration of fibrocytes (per microlitre of blood) (P <
0.05) than SA patients or healthy controls (Figure 1A). Since
fibrocytes are derived from monocytes, particularly from CD14+
monocytes,4–6 we also calculated the ratio of fibrocytes to mono-
cyte population (from full blood count results obtained from
pathology service) or CD14+ monocytes (from flow cytometry). While
the former was reduced in acute MI compared with SA patients or
healthy controls (Figure 1B, P < 0.05), the latter did not differ signifi-
cantly among the three groups (Figure 1D).

3.3 Impaired ability of PBMCs from MI patients to differentiate into fibrocytes
Following 2 weeks’ culture of PBMCs, the morphology of the cultured
cells from the three groups was microscopically examined. The

Figure 1 Measurement of circulating fibrocytes. (A) Representative flow cytometric analysis of circulating fibrocytes: (a) total cells acquired; (b) CD45+ cells; (c) isotype control for collagen I and CD34 set on CD45+ cells; (d) positive staining for collagen I and CD34 set on CD45+ cells;
(e) CD14+ monocytes determined by CD14 PE staining. (B) Circulating fibrocytes in patients with acute myocardial infarction (MI, n = 22), patients with stable angina (SA, n = 20) and healthy controls (Ctrl, n = 22). The percentage of fibrocytes relative to white blood cells (WBC, i.e. CD45+) (a); the concentration of fibrocytes per microlitre of blood (b), the ratio of fibrocytes to monocyte population (from full blood count results obtained from pathology service) (c) or to CD14+ monocytes (d) *P < 0.05, **P < 0.01.
number of spindle-shaped fibrocytes per field under ×40 was counted. A decreased number of fibrocytes were observed in MI and SA patients compared with healthy controls (Figure 2A, *P < 0.05). Using flow cytometry (CD45/collagen I) to identify fibrocytes after culture [since cultured cells lost CD34 expression after 2 weeks (Figure 2B)], we observed that acute MI patients had a reduced proportion of CD45+/collagen I+ fibrocytes of the remaining adherent cells in culture compared with the other two groups (Figure 2C, *P < 0.05).

### 3.4 Decreased gene expression of collagen I and CXCR4 in MI

In addition, the gene expression of collagen I and CXCR4 of these cultured cells was also down-regulated in patients with acute MI compared with healthy controls (Figure 3A and B, *P < 0.05). There was no significant difference in the gene expression of vimentin and fibronectin (Figure 3C and D) among the 3 groups. Notably, CCR7 was very weakly expressed in these cells (data not shown).

### 3.5 Preferential differentiation of mononuclear cells from MI patients into macrophages

Next, we compared the ability of mononuclear cells to differentiate into macrophages. Following stimulation of PBMCs with M-CSF 50 ng/mL for 72 h, expression of CD68, CD36, and EMR was significantly increased in acute MI patients compared to healthy controls (Figure 4, *P < 0.05). CD68 expression was significantly increased in acute MI patients compared with SA patients (*P < 0.05), but...
differences in CD36 and EMR between acute MI and SA patients did not reach statistical significance (Figure 4B and C).

### 3.6 Differences in fibrocyte numbers between SA patient subgroups

SA patients with or without prior MI were also examined as two subgroups (n = 10/group). Baseline characteristics (including smoking status) did not differ significantly between these two subgroups (data not shown). SA patients with previous MI had a lower concentration of fibrocytes per microtitre of blood (P < 0.05, Figure 5A) and a lower percentage of fibrocytes relative to either whole population of WBC (CD45+) or monocyte population than those without previous MI (P < 0.01, P < 0.01 respectively) (Figure 5Ab and c). SA patients with previous MI also had a low yield of fibrocytes from PBMCs after 2 weeks' culture than those without previous MI, as indicated by a decreased number of spindle-shaped fibrocytes or a decreased percentage of CD45+/collagen 1+ fibrocytes in the remaining adherent cells in culture (Figure 5Ba and b, P < 0.05).

### 3.7 Changes in plasma cytokines and chemokines

IL-4 and IL-13 levels were not detected in most plasma samples (data not shown). IFN-γ was detected in 61.5% of plasma samples, which was decreased in acute MI patients compared to healthy controls (Figure 6A). LTE4 (Figure 6H) and IP-10 (Figure 6F) levels were significantly elevated in acute MI patients compared with healthy controls and SA patients (P < 0.05), but there were no significant differences in levels of IL-12, SAP, TGF-β, CD40L, LTC4, cysLTs, SDF, SLC and MCP-1 among the 3 groups (Figure 6B–E, G, I–L). Compared with SA patients without MI, SAP levels were significantly lower in those
with previous MI (43.63 ± 3.45 vs. 56.03 ± 5.34 μg/mL, P < 0.05), but other cytokines/chemokines did not differ significantly between two SA subgroups (data not shown).

4. Discussion

The fibrocyte is a novel cell type that has been shown to participate in wound healing and various fibrotic disorders. The identification of fibrocytes co-expressing pro-collagen I and CD34 in the fibrous cap of human atherosclerotic plaques suggests that fibrocytes may contribute to ECM deposition of the fibrous cap. This prompted us to investigate whether MI was associated with a decreased number of fibrocytes. Here we report for the first time that patients with acute or prior MI have decreased circulating fibrocytes. Furthermore, PBMCs from these patients display preferential differentiation into macrophages rather than fibrocytes compared with healthy controls or SA patients without previous MI. These findings help to advance our understanding of the pathogenesis of atherosclerotic plaque instability. Our work also sheds some light on a future research area investigating the potential use of fibrocytes as a therapeutic target or biomarker in patients with MI.

While accumulating evidence suggests that excessive fibrocytes contribute to fibrotic disorders, our results suggest that reduced fibrocytes numbers may be related to atherosclerotic plaque instability. We found a lower number of circulating fibrocytes in acute MI patients compared with SA patients or healthy controls. After 2 weeks' culture, the ability of PBMCs to differentiate into fibrocytes was also impaired in acute MI patients, as indicated by a reduced spindle-shaped cell count, and a lower percentage of CD45+/collagen I+ fibrocytes and down-regulated gene expression of collagen I and CXCR4 (another marker for fibrocytes) in the remaining adherent cells. In addition, SA patients

Figure 5 Difference in fibrocytes between stable angina (SA) patients with or without previous myocardial infarction (MI) (n = 10/group). (A) Comparisons of circulating fibrocytes between two subgroups, expressed as the concentration of fibrocytes per microlitre of blood (a) and the percentage of fibrocytes relative to either white blood cells (WBC) (b) or monocyte population (from full blood count results obtained from pathology service) (c) or CD14+ monocytes (d). (B) Comparisons of the yield of fibrocytes from PBMCs after 2 weeks' culture between the two subgroups, as measured by spindle-shaped fibrocyte count per field under microscope (a) and the percentage of CD45+/collagen I+ fibrocytes relative to the remaining adherent cells by flow cytometry (b). *P < 0.05, **P < 0.01.
with prior MI displayed a decreased number of circulating fibrocytes and a lower yield of fibrocytes from PBMCs. These data would support that a decreased number of fibrocytes might contribute to thin fibrous caps due to insufficient collagen deposition, consequently contributing to plaque rupture. Currently, there are no ideal predictive biomarkers for unstable plaque and the occurrence of MI. The reduction in the number of circulating fibrocytes might serve as a potential biomarker for plaque instability, but further studies are required to evaluate whether fibrocyte number can predict the occurrence of acute coronary syndrome in SA patients in large-scaled studies.

In acute MI patients, the decrease in the number of circulating fibrocytes may be due to their increased migration into the ruptured sites of atherosclerotic plaques. This is, however, unlikely to be the case as blood samples were obtained immediately upon admission to the hospital, when inflammation and healing responses were thought not to be fully triggered. In addition, the observation that SA patients with previous MI had a decreased number of circulating fibrocytes
compared with those without previous MI suggests that the decrease in circulating fibrocytes is not due to an acute response.

Previous studies report that some healthy individuals have a lower number of fibrocytes produced by PBMCs compared with others, suggesting there is heterogeneity with respect to the ability of PBMCs to differentiate into fibrocytes in culture. Furthermore, adding conditioned medium from PBMC with a high yield of fibrocytes into PBMCs with a low yield of fibrocytes did not increase fibrocyte number, indicating that the low yield of fibrocytes developed from PBMCs in some individuals may be due to the intrinsic property of their monocytes. In this study, we did not observe significant changes in the majority of cytokines and chemokines in plasma that mediate fibrocyte differentiation (IL-12, SAP, TGF-β, IL-4, IL-13, CD40L, LTC4, cysLTs) and migration (SDF, SLC, MCP-1) among the three groups, which may support the notion that deficiency in fibrocytes in patients with MI is likely due to an intrinsic defect in monocyte differentiation into fibrocytes rather than due to external factors. We speculate that monocyte phenotypes may be pre-programmed to adopt their ultimate fate, perhaps via genetic or epigenetic changes in progenitor cells, which warrants further investigation. However, we did observe decreased IFN-γ and increased LTE4 and IP-10 in acute MI patients compared with controls. IFN-γ is known to exert inhibitory effect on fibrocyte differentiation, so the decrease in IFN-γ may serve as a compensatory mechanism for fibrocyte deficiency in patients with acute MI. Vannella et al. found that cysLTs induces proliferation of fibrocytes in a dose-dependent manner. CysLTs measured in this study, which includes LTC4, LTD4, and LTE4, did not significantly change among the three groups on the whole. LTE4 was significantly increased in acute MI patients compared with the other two groups, but the effect of LTE4 alone on the fibrocytes has not been reported. The only observation for the comparisons of plasma cytokines/chemokines between the two subgroups of SA patients is that the SAP level was lower in SA patients with previous MI than those without previous MI, which may also be a compensatory mechanism for a decreased number of fibrocytes in the former. Notably, the plasma level of IP-10 was significantly higher in patients with acute MI compared with healthy controls and SA patients. IP-10 may have antifibrotic effects. Following MI, the early increase in IP-10 prevents premature angiogenesis and fibrous tissue deposition before the area has been cleared of debris.

On the other hand, monocytes are established circulating precursors of tissue macrophages and ruptured plaques have an increased number of macrophages in the fibrous cap compared with unruptured plaques. Our previous studies have shown that PBMCs are activated and their production of MMP-9 is enhanced in acute MI. In this study, we further demonstrated increased expression of macrophage markers on M-CSF stimulated PBMCs from acute MI compared with those from healthy controls. So, the preferential differentiation of PBMCs into macrophages rather than fibrocytes exists in acute MI patients. An increased number of macrophages produce MMPs particularly MMP-9, which digest matrix proteins and weaken the fibrous cap. Therefore, enhanced ECM degradation due to increased macrophages, together with impaired ECM deposition due to a decreased number of fibrocytes, could consequently cause plaque instability.

In conclusion, patients with acute or prior MI are associated with a decreased number of circulating fibrocytes and impaired ability of PBMCs to differentiate into fibrocytes. PBMCs from patients with acute MI show preferential differentiation into macrophages. Further research is needed to investigate the potential use of fibrocytes as a therapeutic target or biomarker in patients with MI.

**Supplementary material**

Supplementary material is available at Cardiovascular Research online.

**Conflict of interest:** none declared.

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