Platelet-Activating Factor Receptor Mediates Oxidized Low Density Lipoprotein-Induced Migration of Bone Marrow-Derived Mesenchymal Stem Cells

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Key Words

Platelet-activating factor • Oxidized low density lipoprotein • Mesenchymal stem cells • Migration • Chemotaxis

Abstract

Background: Oxidized low density lipoprotein (oxLDL) is involved in the development of vascular diseases. Platelet-activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is a key component of oxLDL. **Methods:** In the present study, we evaluate whether oxLDL can regulate migration of human bone-marrow derived stem cells (hBMSCs) and characterize the role of PAF in the oxLDL-induced migration. **Results and Conclusions:** oxLDL stimulated chemotaxis of hBMSCs *in vitro*. Treatment of the cells with BN52021, a specific antagonist of PAF receptor (PAF-R), completely blocked the cell migration induced by PAF, but not platelet-derived growth factor (PDGF-BB). Using PAF-R-specific small interfering RNA, it was demonstrated that silencing of endogenous PAF-R expression significantly attenuated cell migration induced by PAF, but not PDGF-BB, suggesting the specific involvement of PAF-R in the oxLDL-induced cell migration. In addition, PAF-induced migration of hBMSCs was

abrogated by pretreating cells with mitogen-activated protein kinase (MAPK) inhibitors, including the MEK inhibitor U0126, the p38 MAPK inhibitor SB202190, and the JNK inhibitor SP600125. Moreover, adenoviral overexpression of a dominant negative mutant of p38 MAPK blocked PAF-stimulated migration. Taken together, these results suggest that PAF plays a pivotal role in the oxLDL-induced recruitment of hBMSCs through mechanisms involving PAF-R-dependent activation of MAPKs.

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Introduction

Atherosclerosis is a chronic inflammatory disease [1]. Intimal retention and oxidative modification of low density lipoprotein (LDL) play a pivotal role in the development of atherosclerosis [2]. Oxidized LDL (oxLDL) is incorporated into macrophages, leading to transformation of macrophages to foam cells and atherosclerotic plaque formation [3]. In addition, oxLDL reportedly induces oxidative injury in endothelial cells, smooth muscle cells, and macrophages, resulting in atherosclerotic progression [4]. OxLDL has been

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suggested to be involved in various atherogenic steps in the vascular wall such as endothelial dysfunction, migration of smooth muscle cells, and secretion of inflammatory cytokines [1, 2]. However, the molecular mechanism associated with oxLDL-induced cell migration remains elusive.

Oxidation of LDL leads to the formation of numerous oxidized lipid species including platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) from phospholipids [5]. OxLDL reportedly contains PAF-like phospholipid activating vascular cells or immune cells [6, 7]. Under normal physiological conditions, PAF is minimally produced; however, it is abundantly produced under inflammatory conditions associated with atherosclerosis and tissue injury [8]. PAF is associated with cardiovascular pathophysiology, including atherosclerosis, cardiac dysfunction, and myocardial ischemia-reperfusion injury [9]. PAF reportedly stimulates migration of various cell types, including smooth muscle cells, endothelial cells, and cancer cells [10-12]. The PAF effects are mediated through a G protein-coupled PAF receptor (PAF-R) [13]. Consistent with the diverse cellular responses associated with PAF, activation of the PAF-R stimulates numerous signal transduction pathways including phospholipase C, D, A₂ and mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK) and p38 MAPK [8, 13].

Mesenchymal stem cells (MSCs) possess self-renewal capacity, long-term viability, and differentiation potential toward diverse cell types, such as adipogenic, osteogenic, chondrogenic, and myogenic lineages [14]. MSCs can be isolated from a variety of tissues, including bone marrow, adipose tissue, peripheral blood, articular cartilage and synovial tissue [14-16]. MSCs have the potential to differentiate to smooth muscle cells [17-19], suggesting a potential role as progenitor cells for vascular smooth muscle cells. Recent evidence indicates that MSCs are also present in normal artery wall and microvessels [20, 21]. An increasing body of evidence supports the potential involvement of MSCs in the development of atherogenesis and vascular remodeling [22]. However, it is still unclear whether oxLDL can regulate the migration of MSCs.

The present study was undertaken to examine the effects of oxLDL on cell migration of human bone marrow-derived mesenchymal stem cells (hBMSCs). The results demonstrate that PAF mediates the oxLDL-induced migration of hBMSCs through signaling pathways involving MAPKs.

Materials and Methods

Materials

α -Minimum essential medium (α -MEM), trypsin, fetal bovine serum (FBS), and Lipofectamine™ 2000 reagent were purchased from Invitrogen (Carlsbad, CA). U0126, SP600125, and SB202190 were from BIOMOL (Plymouth Meeting, PA). Anti-ERK, anti-phospho-ERK, anti-c-Jun N-terminal kinase (JNK), anti-phospho-JNK, anti-p38 and anti-phospho-p38 antibodies were from Cell Signaling Technology (Beverly, MA). Peroxidase-labeled secondary antibodies and the enhanced chemiluminescence (ECL) kit were purchased from Amersham Biosciences (Franklin Lakes, NJ). Human platelet-derived growth factor-BB (PDGF-BB) was purchased from R&D Systems (Minneapolis, MN). PAF and LDL were purchased from Sigma-Aldrich (St. Louis, MO).

Cell culture

After informed consent, heparinized bone marrow cells were obtained from different individuals undergoing total hip arthroplasty and hBMSCs were isolated as previously described [23]. Briefly, mononuclear cells from bone marrow were separated by centrifugation in a Ficoll-Hypaque gradient (density = 1.077 g/cm³; Sigma-Aldrich), and seeded at a concentration of 1 × 10⁶ cells/cm². Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ in a growth medium comprised of α -MEM, 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μ g/ml of streptomycin until cell growth reached confluence. The primary hBMSCs were subcultured in tissue culture dishes at a concentration of 2,000 cells/cm². The hBMSCs were c-kit, CD34, and CD45 negative and greater than 90% CD29, CD44, CD90, and CD105 positive were used in the experiments.

Lipoprotein preparation

Native LDL (Sigma) was oxidized by exposure to 5 μ M CuSO₄ in PBS at 37°C for 24 hours [24]. The concentration of LDL protein was determined using Bradford protein assay reagent (Bio-Rad). All LDL or oxLDL concentrations used in the experiments were normalized to this protein concentration. For oxidation, LDL was dialyzed against sterile PBS and 10 μ M diethylenetriamine pentaacetic acid (DTPA) and incubated with oxidant 25 μ M CuSO₄ for 14–16 h at 37°C. The oxidation reaction was stopped by the addition of 100 μ M DTPA. Oxidative modification was verified as enhanced mobility by use of agarose gel electrophoresis.

Cell migration assay

Migration of hBMSCs was assayed using a Boyden chamber apparatus, as previously described [25]. Briefly, hBMSCs were harvested with 0.05% trypsin containing 0.02% EDTA, and suspended in α -MEM at a concentration of 2 × 10⁵ cells/ml. Membrane filters (8- μ m pore size) in disposable 96-well chemotaxis chambers (Neuro Probe, Gaithersburg, MD) were pre-coated overnight with 20 μ g/ml rat-tail collagen at room temperature. Aliquots (50 μ l per well) of the cell suspension were loaded into the upper chambers, and test reagents were placed in the lower chamber, unless otherwise specified. To

Fig. 1. Effects of oxLDL and LDL in migration of hBMSCs. (A) hBMSCs were loaded into the upper chambers of the Boyden apparatus and serum-free medium containing the indicated concentrations of oxLDL or LDL were placed in the lower chambers. The numbers of migratory hBMSCs were determined after 12 h as described under “Materials and Methods”. Data represent mean $\pm$ S.D. (n=4). *, $p < 0.01$ vs LDL. (B) Checkerboard analyses of the oxLDL-induced migration. oxLDL (2 μ g/ml) or vehicles was placed in either the bottom, top, or both chambers of the Boyden apparatus, and cells were allowed to migrate for 12 h. Data represent mean $\pm$ S.D. (n=4). * indicates $p < 0.01$.

elucidate the signaling pathways involved in PAF-induced migration, cells were pre-incubated with pharmacological inhibitors for 15 min before loading. After incubation of the cells with PAF in the absence or presence of the inhibitors for 12 h at 37 °C, the filters were disassembled, and the upper surface of each filter was scraped free of cells by wiping it with a cotton swab. The numbers of cells that had migrated to the lower surfaces of each filter were determined by microscopically counting the cells in four places (x100 magnification) after staining with hematoxylin and eosin. To elucidate whether the PAF-induced cell migration represented chemotaxis (directed migration) or chemokinesis (random migration), checkerboard analysis was performed as follows. hBMSCs were resuspended in α -MEM in the absence or presence of PAF just before they were transferred to the upper chamber, and serum-free α -MEM containing PAF or vehicles was added in the lower chamber for generation of concentration gradients of PAF between the upper and the lower compartments. The number of migrated cells on the lower surface of filters was determined by microscopic counting.

Western blotting

hBMSCs were incubated with serum-free α -MEM for 24 h, and the cells were treated with appropriate conditions, washed with ice-cold phosphate buffered saline (PBS), and then lysed in lysis buffer (20 mM Tris-HCl, 1 mM EGTA, 1 mM EDTA, 10 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4 , 30 mM sodium pyrophosphate, 25 mM β -glycerol phosphate, 1% Triton X-100, pH 7.4). The proteins in the lysates were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a nitrocellulose membrane, and stained with 0.1% Ponceau S solution (Sigma-Aldrich) to ensure equal loading. After blocking with 5% nonfat

milk, the membranes were immunoblotted with primary antibodies, and the bound antibodies were visualized with horseradish peroxidase-conjugated secondary antibodies using the ECL Western blotting system (ECL, Amersham Biosciences).

Reverse transcription-polymerase chain reaction (RT-PCR)

Cells were treated as indicated, and total cellular RNA was extracted by the Trizol method (Invitrogen). For RT-PCR analysis, aliquots of 2 μ g RNA were subjected to cDNA synthesis with 200 U of M-MLV reverse transcriptase (Invitrogen) and 0.5 μ g of oligo(dT) 15 primer (Promega, Madison, WI). The cDNA in 2 μ l of the reaction mixture was amplified with 0.5 U of GoTaq DNA polymerase (Promega) and 10 pmol each of sense and antisense primers as follows: PAF-R, sense 5'-TAC TGC TCT GTG GCC TTC CT-3', anti-sense 5'-CTG CCC TTC TCG TAA TGC TC-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sense 5'-TCC ATG ACA ACT TTG GTA TCG-3', antisense 5'-TGT AGC CAA ATT CGT TGT CA-3'. The thermal cycle profile was as follows: denaturation at 95 °C for 30 s, annealing at 53-55 °C for 45 s depending on the primers used, and extension at 72 °C for 45 s. Each PCR reaction was carried out for 30 cycles, and PCR products were size-fractionated on 1.2% ethidium bromide/agarose gel and quantified under ultraviolet transillumination.

Transfection with small interfering RNA

Small interfering RNA (siRNA) duplexes for PAF-R (LQ-005709-00) and non-specific control siRNA (D-001206-13-05) were purchased from Thermo Fisher Scientific. (Dharmacon, IL). For siRNA experiments, hBMSCs were seeded on 60-mm-diameter dishes. When growth reached 70% confluence, cells

Fig. 2. Effects of PAF in the migration of hBMSCs. (A) hBMSCs were loaded into the upper chambers of the Boyden apparatus and serum-free medium containing the indicated concentrations of PAF were placed in the lower chambers. The numbers of migratory hBMSCs were determined after 12 h as described under “Materials and Methods”. (B) Serum-free media containing 2 µg/ml PAF or vehicles (w/o) were added into the lower chambers and the number of hBMSCs migrated to the lower surface of filters was determined after the indicated time periods. (C) Checkerboard analyses of the PAF-induced migration. 2 µg/ml PAF or vehicles was placed in either the bottom, top, or both chambers of the Boyden apparatus as noted, and cells were then allowed to migrate for 12 h. Data represent mean $\pm$ S.D. ($n=4$). * indicates $p<0.01$. (D) hBMSCs attached to the lower surfaces of each filter were photographed by a digital camera mounted on a microscopy (x200 magnification) after staining with hematoxylin and eosin (scale bar = 100 nm). Magnified images are shown as insets (x500 magnification; scale bar = 50 nm) and migrated hBMSCs are indicated by arrows.

were transfected with siRNAs using Lipofectamine Plus™ reagent (Invitrogen) according to manufacturer’s instructions. Briefly, reagent was incubated with serum-free medium for 15 min, and respective siRNAs were then added to the mixtures. After incubation for 15 min at room temperature, the mixtures were diluted with serum free medium and added to each well. The final concentration of siRNAs in each well was 100 nM. After incubation of hBMSCs in serum-free medium containing siRNAs for 4 h, the cells were cultured in growth medium for 24 h, and the expression levels of PAF-R and GAPDH were determined by RT-PCR analysis.

Adenoviral expression of a dominant negative p38 MAPK mutant

The gene encoding dominant-negative mutant of p38 MAPK (TY>AF) [26] was kindly provided by Dr. J. Han (The Scripps Research Institute, La Jolla, CA) and used for production

of recombinant adenoviruses using the AdEasy system (Stratagene, La Jolla, CA). Recombinant adenoviruses were amplified and purified by density gradient ultracentrifugation according to the manufacturer’s manual. For adenoviral infection, exponentially growing hBMSCs were infected with the appropriate amount of adenoviruses and incubated at 37 °C for 2 h with gentle shaking. Afterwards, fresh growth medium was added to each dish and further incubated for 48 h. The expression levels of the dominant negative mutant of p38 MAPK (DN-p38 MAPK) was determined by Western blot analysis.

Statistical analysis

The results of multiple observations are presented as means $\pm$ SD. Statistical significance was assessed using ANOVA and Scheffe’s *post hoc* test, where indicated in the figure legends.

Fig. 3. Role of PAF receptor in the oxLDL-induced chemotaxis of hBMSCs. (A) hBMSCs were pretreated with indicated concentrations of BN52021 for 15 min, loaded into the upper chamber, and serum-free medium containing vehicles (w/o) or 2 μg/ml were added to the lower chambers. (B) hBMSCs were pretreated with 10 μM BN52021 or vehicles (w/o) for 15 min before loading into the upper chamber and serum-free medium containing 2 μg/ml PAF, 2 μg/ml oxLDL, 2 μg/ml LDL, or 10 ng/ml PDGF-BB were added to the lower chamber. (C) hBMSCs were transfected with either control siRNA (si-control) or PAF-R-specific siRNAs (si-PAF-R) and the mRNA levels of PAF-R and GAPDH were determined by RT-PCR. (D) The siRNA-transfected hBMSCs were exposed to serum-free media containing 2 μg/ml PAF, 2 μg/ml oxLDL, 2 μg/ml LDL, or 10 ng/ml PDGF-BB. (E) hBMSCs were treated with a combination of 2 μg/ml PAF and 2 μg/ml oxLDL. The number of migrated cells to lower surface of the filters was determined after 12 h. Data represent mean ± S.D. (n=4). *, p<0.01 by two-way ANOVA and Scheffe's *post hoc* test.

Results

OxLDL- induced migration of hBMSCs

To explore whether oxLDL can regulate migrating ability of hBMSCs, the effects of oxLDL and LDL on cell migration were determined using Boyden chamber apparatus. As shown in Fig. 1A, oxLDL dose-dependently stimulated migration of hBMSCs and the stimulatory effect of oxLDL was more potent than that of LDL. To evaluate whether the oxLDL-induced cell migration was due to chemotaxis (directed migration) or chemokinesis (random migration), "checkerboard" analysis was performed. OxLDL-induced migration occurred in the presence of a positive concentration gradient between two

compartments (higher concentration below the filter). In the presence of equal concentrations of oxLDL above and below the filter or of a negative gradient (higher concentration above the filter), no migration occurred (Fig. 1B), suggesting chemotactic migration of hBMSCs in response to oxLDL.

PAF stimulates migration of hBMSCs

In order to test whether PAF could regulate migrating ability of hBMSCs, the effect of PAF on the migration of hBMSCs was determined. PAF dose-dependently stimulated migration of hBMSCs with a maximal induction at 1 μg/ml (Fig. 2A). Moreover, PAF time-dependently increased migration of hBMSCs and the PAF-induced

Fig. 4. Role of MAPKs in the PAF-induced migration of hBMSCs. (A) hBMSCs pre-incubated in the absence or presence of 10 μ M U0126, 10 μ M SP600125, or 10 μ M SB202190 for 15 min were stimulated with 2 μ g/ml of PAF for 10 min. The phosphorylation and expression levels of ERK, JNK, and p38 MAPK were analyzed by Western blotting. Representative data from three independent experiments are shown. (B-C) hBMSCs were pretreated with vehicles, 10 μ M U0126, 10 μ M SP600125, or 10 μ M SB202190 for 15 min, and then exposed to serum-free medium containing 2 μ g/ml PAF (B) or 2 μ g/ml oxLDL (C) in the absence or presence of the pharmacological inhibitors for 12 h. The number of migrated cells was counted and data represent means $\pm$ S.D. (n=4). * indicates $p < 0.01$ by two-way ANOVA and Scheffe's *post hoc* test.

migration was maximum after exposure of hBMSCs for 12 h (Fig. 2B). The PAF-induced cell migration was mediated by chemotaxis, because cell migration occurred in the presence of PAF in the lower chamber (Fig. 2C), indicating that PAF induces a chemotactic response in hBMSCs with no appreciable chemokinetic activity. hBMSCs normally exhibited fibroblast-like morphology and the morphology of hBMSCs was not apparently changed after PAF-induced chemotactic migration (Fig. 2D).

PAF-R is involved in oxLDL-induced migration of hBMSCs

OxLDL contains PAF and PAF-like phospholipids [27], and PAF-R is responsible for the diverse biological responses of PAF [13]. To explore whether the oxLDL-induced cell migration is mediated by PAF-R, the effects of the PAF-R-specific antagonist BN52021 on PAF-stimulated migration of hBMSCs were examined. As shown in Fig. 3A, BN52021 dose-dependently inhibited the PAF-induced migration of hBMSCs. BN52021 (10 μ M) completely abrogated the cell migration induced by PAF and oxLDL (Fig. 3B). To ensure that BN52021 specifically inhibited PAF-induced migration, we determined the effects of BN52021 on the PDGF-

induced migration of hBMSCs. As shown in Fig. 3B, BN52021 had no significant impact on the PDGF-BB-induced cell migration, suggesting a specific involvement of PAF-R in the migration of hBMSCs induced by oxLDL and PAF.

To confirm the notion that PAF-R is responsible for the oxLDL-induced cell migration, the effects of siRNA-mediated depletion of endogenous PAF-R on cell migration were assessed. As shown in Fig. 3C, mRNA levels of PAF-R in hBMSCs were diminished by transfection with PAF-R-specific siRNAs. PAF-induced migration of hBMSCs was completely abrogated by silencing of endogenous PAF-R expression (Fig. 3D). Furthermore, knockdown of PAF-R blocked the oxLDL-induced cell migration. However, PDGF-BB-induced cell migration was not affected by silencing of PAF-R. Consistent with these results, combined treatment of hBMSCs with saturating concentrations of oxLDL and PAF had no synergy or additive effects on cell migration when compared with either oxLDL or PAF was treated separately, indicating that oxLDL and PAF likely share downstream signaling mechanism (Fig. 3E). Taken together, these observations obviously suggest that PAF-R plays a pivotal role in the oxLDL-induced migration of hBMSCs.

Fig. 5. Role of p38 MAPK in the PAF-induced cell migration. (A) hBMSCs were infected with adenoviruses bearing DN-p38 MAPK or LacZ at a multiplicity of infection (MOI) of 50 plaque forming units (pfu) for 48 h. The expression levels of p38 MAPK were determined by Western blotting. Representative data from three independent experiments are shown. (B-C) The adenoviral infected and uninfected control cells were then exposed to serum-free medium containing 2 μ g/ml PAF (B) or 2 μ g/ml oxLDL (C) for 12 h. The number of migrated cells was counted and data represent means $\pm$ S.D. (n=4). * indicates $p < 0.01$ by two-way ANOVA and Scheffe's *post hoc* test.

PAF induces migration of hBMSCs through MAPK-dependent mechanisms

PAF stimulates MAPKs including ERK and p38 MAPK [8, 13]. Appropriately, an experiment was conducted to determine the phosphorylation levels of three MAPKs, including ERK, JNK, and p38 MAPK, in response to PAF treatment. As shown in Fig. 4A, PAF increased phosphorylation levels of ERK, JNK, and p38 MAPK in hBMSCs. These phosphorylations were abrogated by pretreatment of the cells with U0126, SP600125, and SB202190, respectively. To elucidate the involvement of MAPKs in the PAF-induced migration, the effects of these pharmacological inhibitors on the PAF-induced migration of hBMSCs were determined. As shown in Fig. 4B, the PAF-stimulated migration of hBMSCs was abolished by pretreatment of the cells with U0126, SP600125, or SB202190. Furthermore, oxLDL-induced migration was also abrogated by pretreatment of the cells with U0126, SP600125, or SB202190. These results suggest that ERK, JNK, and p38 MAPK are involved in the migration of hBMSCs induced by PAF and oxLDL.

p38 MAPK plays a key role in PAF-induced migration of hBMSCs

Both ERK and JNK are reportedly implicated in migration of a variety of cell types. However, the role of

p38 MAPK in cell migration is still elusive, in spite of prominent involvement of p38 MAPK in PAF-induced cell signaling [8, 13]. To further support the role of p38 MAPK in PAF-induced migration, the effects of a dominant negative mutant of p38 MAPK (DN-p38 MAPK) on cell migration were examined. DN-p38 MAPK was overexpressed in hBMSCs using adenoviral expression system (Fig. 5A). Overexpression of DN-p38 MAPK blocked the PAF-induced phosphorylation of p38 MAPK. In addition, adenoviral over-expression of DN-p38 MAPK abrogated the migration of hBMSCs induced by either PAF or oxLDL (Fig. 5B and 5C). These results suggest that p38 MAPK is involved in the chemotaxis of hBMSCs induced by PAF and oxLDL.

Discussion

In the present study, we demonstrated that oxLDL potently stimulated migration of hBMSCs through a PAF/PAF-R-dependent mechanism. Migration of hBMSCs induced by PAF and oxLDL was completely abrogated by pre-treatment with the PAF-R-specific antagonist BN52021 or siRNA-mediated depletion of endogenous PAF-R expression. PAF-induced activation of PAF-R stimulates migration of various cell types including

endothelial cells, cancer cells, and a synthetic phenotype of smooth muscle cells [10-12]. OxLDL contains PAF and PAF-like phospholipids [27]. Activation of PAF-R is associated with oxLDL-induced chemokine release and adhesion in monocytes [7, 28]. Furthermore, oxLDL stimulates proliferation of smooth muscle cells through a PAF-R-dependent mechanism [6]. However, oxLDL particles are very heterogeneous in size, composition, and proportion of oxidized components. Therefore, it is difficult to determine the respective part of each constituent in their biological properties. Accumulating evidence suggests that the atherogenic properties of oxLDL are largely attributed to PAF and oxidized phospholipids, such as POVPC, and PGPC [7, 29]. It has been reported that POVPC can activate PAF-R [30], suggesting that not only PAF but also oxidized phospholipids may be responsible for the oxLDL-induced cell migration. We found that POVPC can stimulate migration of hBMSCs through PAF-R-dependent mechanism (data not shown). Taken together, these results support a critical role of PAF-R in oxLDL-induced chemotactic migration of hBMSCs, although molecular identities of the factors associated with oxLDL-induced cell migration should be determined further.

PAF activates ERK in Chinese hamster ovary cells and human neutrophils [13, 31]. Moreover, PAF induces activation of p38 MAPK, ERK, and JNK in airway smooth muscle cells and primary hippocampal neurons [32, 33]. However, the functional involvement of these MAPKs in the PAF-induced cell migration has not been clearly elucidated. Presently, PAF-induced migration of hBMSCs was abrogated by pretreatment with pharmacological inhibitors against ERK, JNK, or p38 MAPK, suggesting involvement of the three MAPK signaling pathways in PAF-induced cell migration. ERK, JNK, and p38 MAPK have been implicated in the regulation of cell migration of diverse cell types [34]. Overexpression of a constitutively active MEK1 mutant promoted the migration of FG carcinoma cells, whereas depletion of endogenous ERK by antisense oligonucleotides inhibited cell migration [35]. Furthermore, p38 MAPK is involved in migration of smooth muscle cells induced by PDGF, transforming growth factor- β , and interleukin-1 β [36], and PDGF-induced migration of endothelial cells is dependent on p38 MAPK activation [37]. Consistently, the present data showed that adenoviral over-expression of DN-p38 MAPK abrogated the PAF-stimulated migration

of hBMSCs. Taken together, these results support the notion that three MAPKs play a central part in the PAF-induced migration of hBMSCs.

MSCs reportedly migrate from bone marrow into injured tissues and tumor tissues, and, thus, play a role in tissue regeneration, fibrosis, and tumorigenesis [14, 38]. It has been documented that MSCs migrate in response to various protein factors, including PDGF, stromal-derived factor-1, and monocyte chemoattractant protein-1 [39-41]. In addition, accumulating evidence suggests that lipid-derived factors such as lysophosphatidic acid and sphingosine-1-phosphate are involved in migration of MSCs [42-44]. The findings of the present study implicate oxLDL-associated PAF as a chemotactic factor for MSCs. It has been shown that human umbilical vein endothelial cells exposed to oxLDL secreted SDF-1 α and paracrine activation of SDF-1 α -CXCR4 signaling axis stimulated migration and adhesion of BMSCs [45]. These results suggest that oxLDL can induce migration of BMSCs through direct activation of PAF-R or indirect activation of SDF-1 α -CXCR4 signaling axis. Because oxLDL plays a key role in the development of atherogenesis, it is conceivable that migrated MSCs play a key role in the development of atherogenesis in response to oxLDL or PAF. In line with this hypothesis, OxLDL has been reported to induce transdifferentiation of BMSC-derived smooth muscle-like cells to foam cells, which play a pivotal role in the development of atherosclerosis [46]. In addition, oxLDL inhibited osteogenic differentiation of BMSCs, whereas adipogenic differentiation of BMSCs was stimulated by oxLDL [47]. These results suggest that oxLDL may induce not only atherosclerosis but also osteoporosis by directing BMSCs to undergo adipogenic instead of osteogenic differentiation. In addition to the role of oxLDL-associated PAF in atherogenesis, PAF is mainly produced in response to inflammatory stimuli during tissue injury [8]. Therefore, it is tempting to speculate that PAF may be involved in the homing of MSCs into injured tissues, although it should be determined further whether PAF is involved in the migration of MSCs *in vivo*.

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