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PEDF From Mouse Mesenchymal Stem Cell Secretome Attracts Fibroblasts

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Abstract Conditioned medium (secretome) derived from an enriched stem cell culture stimulates chemotaxis of human fibroblasts. These cells are classified as multipotent murine mesenchymal stromal cells (mMSC) by immunochemical analysis of marker proteins. Proteomic analysis of mMSC secretome identifies nineteen secreted proteins, including extracellular matrix structural proteins, collagen processing enzymes, pigment epithelium-derived factor (PEDF) and cystatin C. Immunodepletion and reconstitution experiments show that PEDF is the predominant fibroblast chemoattractant in the conditioned medium, and immunofluorescence microscopy shows strong staining for PEDF in the cytoplasm, at the cell surface, and in intercellular space between mMSCs. This stimulatory effect of PEDF on fibroblast chemotaxis is in contrast to the PEDF-mediated inhibition of endothelial cell migration, reported previously. These differential functional effects of PEDF toward fibroblasts and endothelial cells may serve to program an ordered temporal sequence of scaffold building followed by angiogenesis during wound healing. J. Cell. Biochem. 104: 1793–1802, 2008.

Key words: murine marrow stromal cells; secretome; fibroblast chemoattractant; PEDF

Tissue regeneration in adults depends on the presence of functional stem cells, capable of renewing or differentiating into mature cell types [Prockop, 1997; Weissman, 2000]. The fate of an individual stem cell is dictated by its local microenvironment, the stem cell niche [Schofield, 1978; Scadden, 2006], defined by the secreted products of the stem cell. Thus, identification and functional characterization of the proteins secreted by stem cells are essential for understanding these cells' paracrine and autocrine activities.

Bone marrow is a source of both hematopoietic and non-hematopoietic stem cells. It has been recognized since the mid-1970s that non-hematopoietic stem cells from bone marrow adhere to the substratum of culture ware, whereas hematopoietic stem cells are non-adherent [Friedenstein et al., 1970; Prockop, 1997]. These adherent cells, defined as multipotent mesenchymal stromal cells [Horwitz et al., 2005], constitute a heterogeneous population that can be cultured and induced to differentiate into various mature cell types by appropriate stimuli [Grove et al., 2004; Horwitz et al., 2005; He et al., 2007]. Heterogeneous mesenchymal stromal cell populations have been shown to synthesize and secrete a variety of extracellular matrix proteins, including collagen types I and IV, fibronectin and laminin, as well as cytokines, growth factors, and other bioactive proteins [Prockop, 1997].

Our interest in these cells is driven by the hypothesis that specific mesenchymal stromal cells circulating in the blood (e.g., fibrocytes) can migrate to a wound site, and through

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their secretory activity provide a microenvironment that stimulates differentiation and regulates the influx of cell types required for optimal tissue regeneration in a time-dependent manner. To investigate this, we enriched and characterized a cell line from adult mouse bone marrow as stem cell-like, based on its repertoire of signature cell surface markers. We found that the secretome of these cells stimulates migration of two human fibroblast cell lines, WI-38 and IMR-90. We identified various proteins in mMSC secretome by high-resolution, two-dimensional LC MS/MS, and confirmed the presence of selected major proteins by immunoblot and immunohistochemical analysis. We then identified pigment epithelium-derived factor (PEDF), a member of the serpin protease inhibitor family, as the major chemoattractant for fibroblasts in the mMSC secretome.

MATERIALS AND METHODS

Antibodies and Chemicals

Horseradish peroxidase (HRP)-conjugated sheep anti-rabbit IgG was obtained from MP Biomedicals (Solon, OH), HRP-conjugated goat anti-mouse IgG from Pierce Biotechnology (Rockford, IL), rabbit polyclonal anti-PEDF and anti-cystatin C from Upstate (Lake Placid, NY), rabbit polyclonal anti-collagen type I from US Biologicals (Swampscott, MA), and purified rabbit IgG from Calbiochem (La Jolla, CA). The antibodies used for stem cell characterization were: rat anti-human HLA-DR:FITC and mouse anti-mouse MHC class I H-2Db:FITC (AbD Serotec, Raleigh, NC), FITC anti-mouse CD90 (Thy) (Cedarlane Laboratories, Burlington, NC), anti-mouse CD105/Endoglin-fluorescein (R&D Systems, Minneapolis, MN), and biotin conjugated rat anti-mouse Ly-6A/E (Sca-1) (BD Biosciences, San Jose, CA).

PEDF was purified from fetal bovine serum (FBS) (Hyclone, Logan, UT) or human plasma (Innovative Research, Southfield, MI) by affinity chromatography on a collagen-Sepharose 4B column [Petersen et al., 2003]. PEDF was eluted at both 0.2 and 0.5 M NaCl, as assessed by Immunoblot analysis. The identity of the purified human and bovine proteins from both fractions was confirmed by a ToF Spec 2E MALDI-ToF mass spectrometer (Micromass, Manchester, UK), by searching the Uni-ProtKB/Swiss-Prot data base using *Mascot* software (Matrix Science Ltd., London, UK).

Animals

Mice were housed in a barrier animal facility at the University of Louisville, and cared for according to National Institutes of Health animal care guidelines.

Cell Culture

Human lung embryonic fibroblasts (WI-38 and IMR-90) were obtained from the ATCC, and cultured in minimum essential medium (MEM, Hyclone) supplemented with 10% heat-inactivated FBS. Cells at early passage (population doubling levels 30–35) were used for all experiments in this study.

Isolation and Enrichment of Stem Cells

Old mice were used to isolate the adult stem cells. Bone marrow was flushed from tibias and femurs of 28-month-old C57/Bl6 male mice with DMEM containing antibiotics, using a 22-gauge syringe. The marrow was mechanically dispersed to form a single cell suspension, and plated on culture dishes; the cells were maintained at 37°C, 5% CO_2 in an incubator. Subsequently, cells were separated into single cell suspensions by serial dilution. When confluent, the cells were analyzed by incubating with marker proteins Sca-1, CD45, MHC-1, CD90, and CD105. The expression of these antigens was determined with a fluorescence-activated cell sorter (Becton Dickinson, Franklin Lakes, NJ).

Preparation of Stem Cell Secretome

mMSCs were grown to 90% confluency, washed three times with DMEM, and incubated with 4.5 ml of DMEM at 37°C, 5% CO_2 for 42 h. The conditioned medium was collected and centrifuged at 1,000*g* for 10 min at 4°C. The resulting supernatant (defined as the stem cell secretome) was aliquoted, and frozen at −80°C. For immunoblot and transmigration experiments, the conditioned medium was concentrated using Centriplus® centrifugal filter devices (Millipore, Billerica, MA), according to the manufacturer's instructions.

Cell Migration Assays

Cell migration assays were performed using transwell migratory chambers (8 μm pore size, Corning Costar, Cambridge, MA) [Adelmann-Grill and Cully, 1990]. WI-38 and IMR-90 fibroblasts were serum-starved overnight;

5×10^4 cells in 100 μl MEM were seeded in the upper reservoir. MEM containing 10% FBS, MEM, or stem cell secretome was added to the lower reservoir. Cells were allowed to migrate for 24 h at 37°C. Cells that had migrated to the lower side of the membrane were stained with 0.1% crystal violet in phosphate-buffered saline, visualized by microscopy, and counted manually.

LC MS/MS Proteomic Analysis

Proteomic analysis of the secretome was carried out essentially as described by Cong et al. [2006]. Briefly, 25 μg of trypsin was added to 4 ml of secretome, and this mixture was incubated overnight at 37°C. The resulting digest was injected into a BioCAD HPLC system equipped with a reverse phase C18 column (Waters, Milford, MA). The column was washed with buffer A [2% acetonitrile, 0.05% trifluoroacetic acid (TFA, Sigma–Aldrich, St. Louis, MO) in water] at a flow rate of 1 ml/min. Peptides were then eluted by a linear gradient of 0–100% buffer B (0.05% TFA in 100% acetonitrile). Peak fractions were collected, dried, dissolved in buffer A, and desalted using a C18 zip tip (Millipore). Subsequently, the tryptic peptides were processed for identification and quantification by a QSTAR® XL hybrid liquid tandem mass spectrometry system (Applied Biosystems) interfaced with an 1100 Series Capillary LC system (Agilent, Sta. Clara, CA). Individual fractions containing tryptic peptides were injected using a 100 series autosampler (Agilent) into a C-18 analytical reversed phase column (75 μm × 150 mm, Vydac 5 nm C18 polymeric, Alltech, Nicholasville, KY), and eluted using a binary gradient (400 nl/min) from 100% buffer A (2.5% acetonitrile, 0.4% acetic acid, 0.01% heptafluorobutyric acid) to 20% buffer B (0.4% acetic acid, 0.01% heptafluorobutyric acid in 100% acetonitrile) for 20 min, then from 20 to 70% buffer B for 5 min. MS ToF scans were acquired from m/z 350 to 1,600, with up to two precursors selected for MS/MS from m/z 60 to 2,000 using information-dependent acquisition. The rolling collision energy method was used to promote fragmentation. MS and MS/MS data from the QSTAR system were analyzed for protein identification using ProQUANT 1.0 software (Applied Biosystems). Database searching was restricted to tryptic peptides derived from the UniProtKB/Swiss-Prot protein database. Methionine oxidation was selected as variable, one missed cleavage was allowed, precursor error tolerance was set at <0.15 Da, and product ion error tolerance was set at <0.1 Da.

Western Blot Analysis

Cells were harvested with cell scrapers in ice-cold PBS, and centrifuged at 250*g* for 5 min. Cell pellets were resuspended in extraction buffer (20 mM Tris–HCl, pH 7.4, 300 mM NaCl, 2% Triton X-100, 2 mM EDTA, 0.2% SDS) supplemented with protease inhibitors (Calbiochem). The cell suspension was agitated at 4°C for 30 min, followed by centrifugation at 15,000*g* for 20 min. Protein extracts were resolved on 9–12% SDS–PAGE gels, and transferred to nitrocellulose membranes. Subsequently, the membranes were blocked with 5% non-fat dry milk (LabScientific, Livingston, NJ), washed, and incubated overnight with the indicated primary antibodies on a rotary shaker at 4°C. After washing, the blots were incubated with HRP-conjugated second antibody for 1 h at room temperature, followed by incubation with enhanced chemiluminescence reagent (Amersham) for 1 min. Protein bands were visualized by exposure to Kodak X-OMAT film.

RT-PCR Analysis

Total RNA from stem cells, WI-38 and IMR-90 cells in cultures of 70% confluence was prepared using TRIzol reagent (Invitrogen). cDNAs were synthesized using the First-Strand Synthesis Kit (Promega), according to the manufacturer's instructions. PCR reactions were performed in a final volume of 25 μl containing 1 μl of reverse-transcribed cDNA and 10 μM of specific primers carried out at 94°C (20 s), 56°C (30 s), and 72°C (35 s) for 30 cycles. PCR products were resolved on 1.5% agarose gels, and visualized with ethidium bromide staining. The primers used were: PEDF (sense) 5′-CCCTGGTGCTACTCCTCTG-3′, (anti-sense) 5′-TAGCCGAAGTTGGAGACAGC-3′; collagen α1 (sense) 5′-ACGTCCTGGTGAAGTTGGTC-3′, (anti-sense) 5′-GTTTGCCAGGTTCACCAGAG-3′; cystatin-C1 (sense) 5′-ACGAAGTGTACCACAGCCGCGC-3′, (anti-sense) 5′-TCAGATGCGGCTGGTCATGGAA-3′; 18S RNA (sense) 5′-ATGCTCTTAGCTGAGTGGCCCG-3′, (anti-sense) 5′-ATTCCTAGCTGCGGTATCCAGG-3′; and PEDF Receptor (sense) 5′-TCAATGAGGCCCTGCTGGAG-3′ (anti-sense) 5′-TAGGGCACCATCATGGCCGT-3′.

Immunofluorescence Analysis

Mouse mesenchymal stem cells were plated in 35 mm glass bottom dishes (MatTek Corporation, Ashland, MA). After 48 h, the cells were fixed with 4% paraformaldehyde in PBS, and permeabilized with 0.25% Triton-X in PBS. Rabbit anti-PEDF (Upstate) and rabbit anti-myosin antibody (Calbiochem) were used for PEDF and Myosin staining, followed by the second antibody, Alexa 568-conjugated goat anti-rabbit antibody (1:1,000 dilution, Molecular Probes, Invitrogen, Carlsbad, CA). The antibody-associated fluorescence in the cell was imaged with an Axiovert 200 M epi-fluorescence microscope (Carl Zeiss).

PEDF Depletion

Protein A/G agarose beads (Pierce) were cross-linked with anti-PEDF; negative control

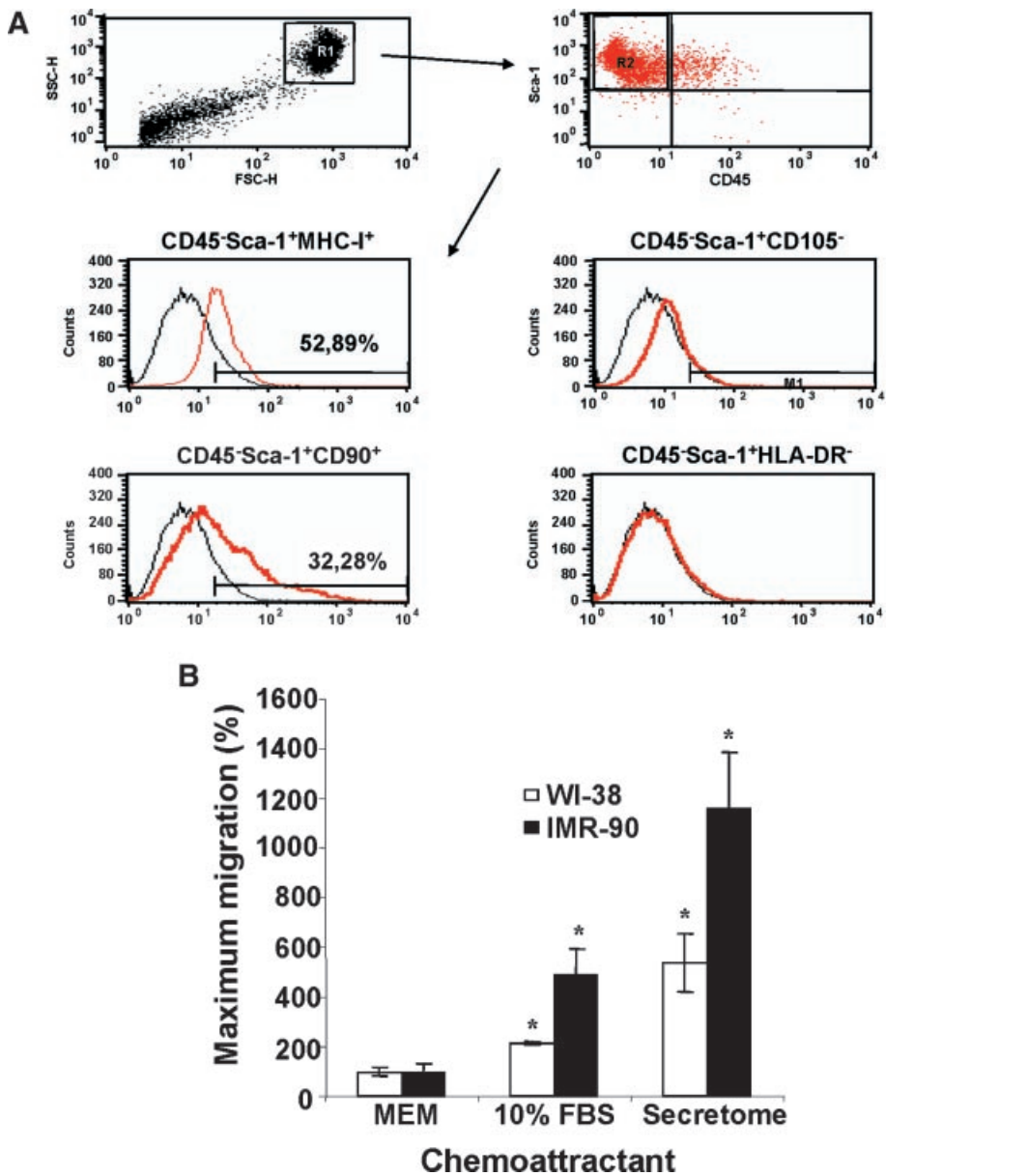

Fig. 1. Characterization of an enriched mouse mesenchymal stem cell (mMSC) line and the mMSC secretome enhances chemotaxis of human fibroblasts. **A**: Enriched mouse bone marrow cells were characterized by flow cytometry, using fluorescent antibodies to specific cell surface markers. This analysis shows that the cells are positive for reactivity toward MHC-1, CD90, and Sca-1, and negative for CD45, HLA-DR, and CD105. **B**: Chemotactic activity is expressed as the percent increase in the number of cells migrating toward the test medium, compared to those migrating toward serum-free medium. Data are the mean ± SD from three independent assays. *Significantly different from the serum-free medium control, $P < 0.05$.

beads were cross-linked with purified rabbit IgG, as previously described [Lee et al., 1999]. An aliquot of the stem cell secretome was incubated with anti-PEDF or IgG beads at room temperature for 1 h. The beads were collected by centrifugation at 1,000*g* for 5 min, and the resulting supernatants were used as chemoattractants in migration assays. PEDF depletion was confirmed by Immunoblot analysis of the supernatant.

RESULTS

To eliminate cellular heterogeneity of stem cells, mMSC samples were enriched with bone marrow cells of male C57/Bl6 mice. Single cells were chosen for expansion in serum-containing medium. When the cells became confluent, the cellular phenotype was characterized by the presence of characteristic surface antigens by flow cytometry. As shown in Figure 1A, the selected population (R1) is gated (R2) for Sca-1^+ $CD45^-$ (90.80%). Hemopoietic cells from species such as mice coexist in culture with mesenchymal stem cells, even after several passages [Baddoo et al., 2003; Javazon et al., 2004]. From the Sca-1+ and $CD45^-$ cell population, 52.9% expressed $CD45^-$Sca-1 + MHC-I+, and 32.3% expressed $CD45^-$Sca-1 + CD90+. No cells expressed $CD45^-$Sca-1 + $CD105^-$ or HLA-DR^-. However, it has been reported that the expression of many markers on mesenchymal stem cells is variable [Jones et al., 2002; Baddoo et al., 2003; Javazon et al., 2004; Peister et al., 2004]. Multipotent mesenchymal stem cells express, and are typically positive for Sca-1, and negative for CD45 [Martin et al., 2002; Sun et al., 2003; Javazon et al., 2004]. Thus we conclude that the enriched stem cells used in this study are representative of the mMSC population.

We next showed that the secretome of this mMSC is chemotactic for human fibroblasts. Figure 1B shows that mMSC conditioned medium markedly stimulates migration of WI-38 and IMR-90 human fibroblasts; IMR-90 cells exhibit a stronger response than WI-38. Moreover, the mMSC secretome is more effective than serum-supplemented medium.

To identify candidate proteins responsible for the observed fibroblast chemotactic activity, we carried out a high-resolution proteomic analysis of mMSC conditioned medium, using LC MS/MS [Cong et al., 2006]. Nineteen proteins in the mMSC secretome, identified with >95% certainty, are known to be secreted based on criteria in the UniProtKB/Swiss-Prot data base (http://us.expasy.org/sprot/); they are summarized in Table I. Structural proteins, such as collagen type I and fibronectin, have previously been shown to be secreted by other mMSC lines; proteins such as PEDF and cystatin C have not.

TABLE I. Secreted Proteins Identified by Proteomic Analysis of mMSC-Conditioned Medium

Swiss prot accession #	Name	Confidence	# of peptides identified
P11276	Fibronectin precursor	99	24
P97298	Pigment epithelium-derived factor precursor (PEDF)	99	21
Q01149	Collagen α2(I) chain precursor	99	20
O70624	Myocilin precursor	99	10
Q06890	Clusterin precursor	98	7
P28301	Protein-lysine 6-oxidase precursor	99	7
P28653	Biglycan precursor	99	6
P06797	Cathepsin L precursor	99	6
P17742	Peptidyl-prolyl cis-trans isomerase α	99	6
Q02819	Nucleobindin 1 precursor	99	5
Q61398	Procollagen c-proteinase enhancer protein precursor	99	5
P11087	Collagen α1(I) chain precursor	99	3
Q9QUN9	Dickkopf related protein-3 precursor (Dkk-3)	99	3
P37889	Fibulin-2 precursor	99	3
P01887	β-2-microglobulin precursor	99	2
P12399	CTLA-2-alpha protein precursor	95	2
P21460	Cystatin c precursor	99	2
P16110	Galectin-3	99	1
P26041	Moesin	99	1

Proteins were classified as "secreted" based on analysis of the complete set of proteins using functional annotations in the UniProtKB/Swiss-Prot sequence database (http://us.expasy.org/sprot/).

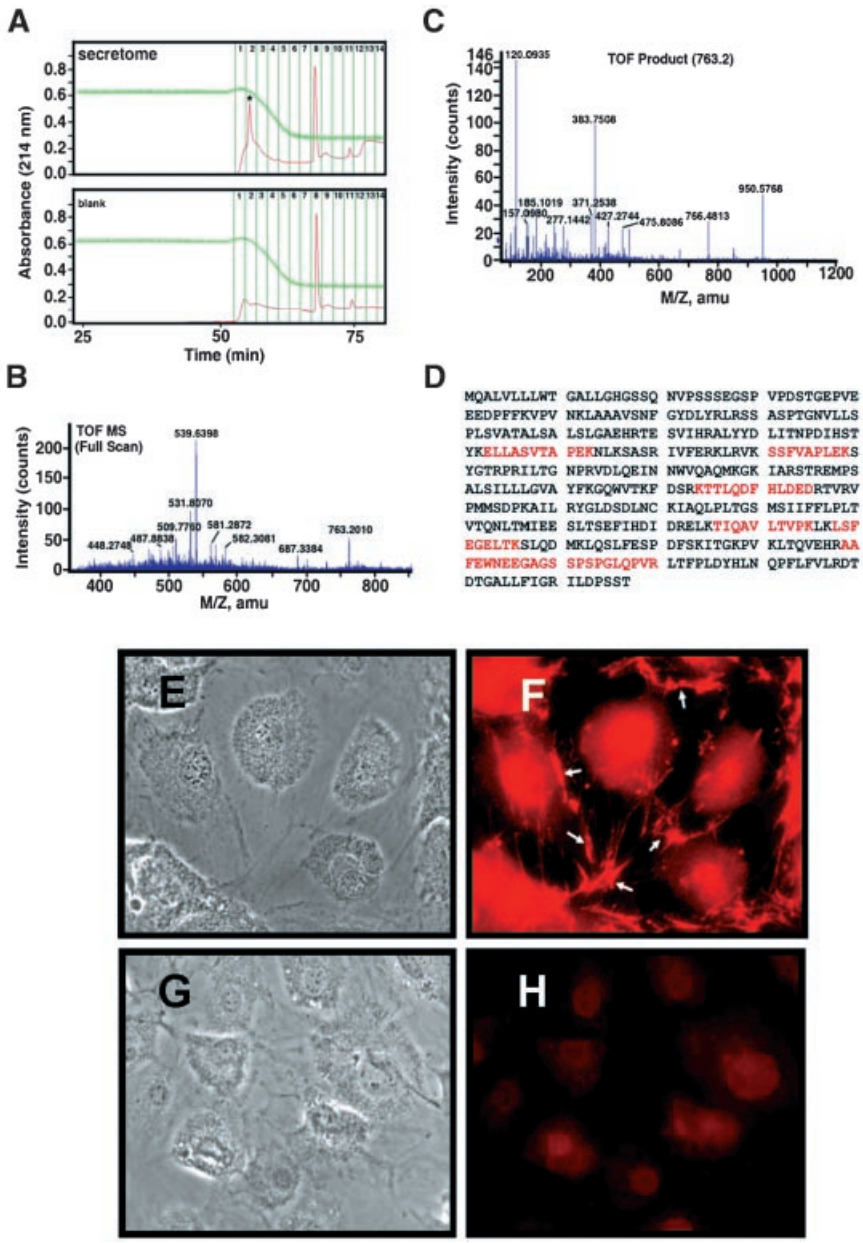

Fig. 2. LC MS/MS identification of PEDF in the mMSC secretome and the expression of PEDF by mouse mesenchymal stem cells. **A**: Reverse phase HPLC profile of an overnight tryptic digest of the mMSC secretome. **B**: Full scan (TOF) mass spectrum of fraction 2 from the HPLC separation shown in (A). **C**: TOF fragmentation spectrum of the mass peak at 763.20 *m*/*z* in (B). The 763.20 *m*/*z* peak in panel (B) corresponds to the triply charged peptide AAFEWNEEGAGSSPSPGLQPVR in **panel D** (expected $m/z = 2285.06$). **D**: Amino acid sequence of mouse PEDF (UniProtKB/Swiss-Prot entry P97298). PEDF peptides identified by MS/MS are shown in red. **E**: The **panel F** shows the localization of PEDF by fluorescence microscopy. The **panel H** is negative control showing stem cells stained with an antibody to myosin, which was shown by RT-PCR to be of low abundance (data not shown). **Panels E** and **G** are corresponding phase contrast photomicrographs.

One of the most abundant proteins identified in mMSC conditioned medium is PEDF. Figure 2A depicts the HPLC profile of a tryptic digest of mMSC conditioned medium, while Figure 2B,C shows the mass spectra associated with identification of one tryptic peptide. The complete coverage of identified peptides within the sequence of mouse PEDF is given in panel 2D.

Localization of PEDF in cultured mMSCs was determined by fluorescence microscopy. Figure 2F shows intense staining by anti-PEDF in the rough endoplasmic reticulum/Golgi areas, which demonstrates that PEDF is

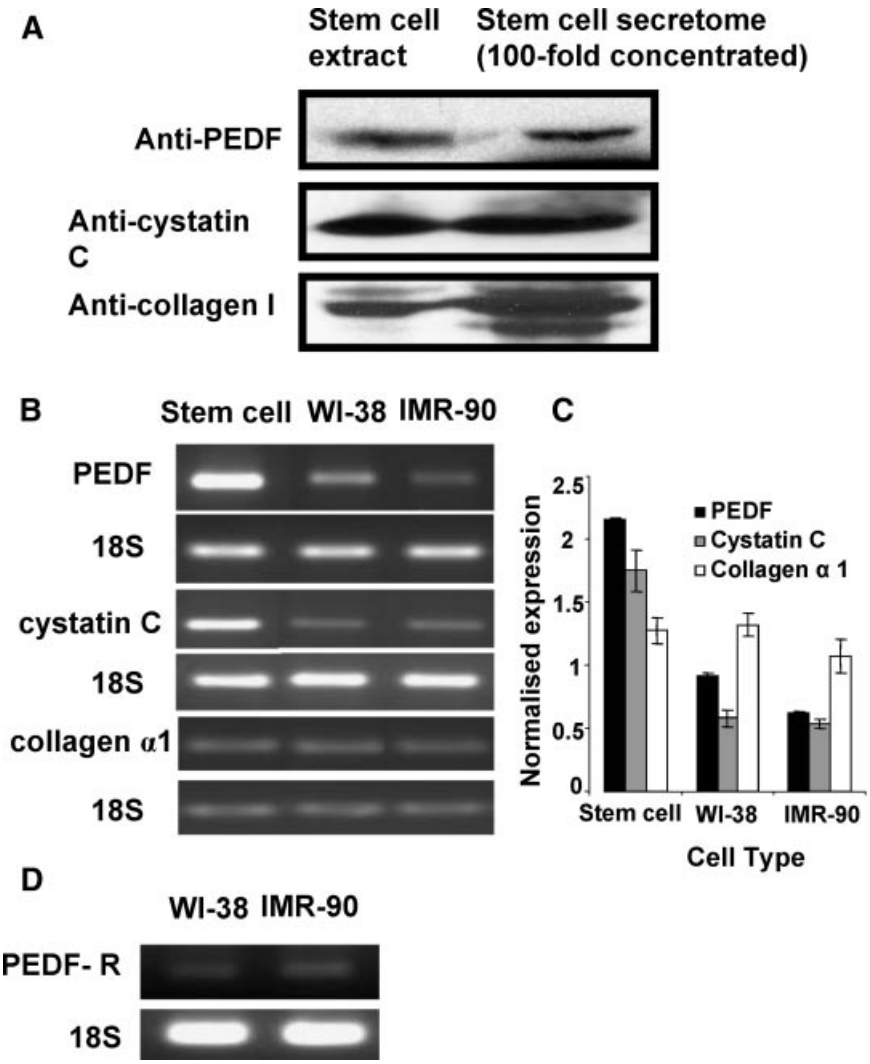

Fig. 3. Immunochemical validation of selected mMSC secretome proteins identified by MS/MS, and expression levels of transcripts in stem cells and fibroblasts. **A**: Western blots of mMSC cellular extracts (**left**) and secretome (**right**). **B**: RT-PCR analysis of mRNA transcripts of PEDF, cystatin C, collagen α1 (I), and 18S RNA. **C**: The expression levels of respective mRNA in mMSCs, WI-38, and IMR-90 fibroblasts were determined by densitometry, and normalized to 18 S RNA (n = 3). **D**: RT-PCR analysis of mRNA transcripts of PEDF receptor in RNA from extracts of WI-38 and IMR-90 cells.

robustly synthesized in mMSCs. PEDF is also found to be located near the plasma membrane and in the extracellular space (arrows, Fig. 2F), consistent with the known ability of PEDF to bind to collagen and proteoglycan in the extracellular matrix [Meyer et al., 2002; Yasui et al., 2003]. To assess non-specific staining, negative control immunofluorescence experiments were performed with antibodies to a low-abundance protein, myosin (Fig. 2H), and with second antibody alone (data not shown).

The mass spectroscopic data were validated to confirm the presence of selected proteins by Immunoblot analysis. PEDF, collagen α (I), and cystatin C polypeptides were clearly evident in mMSC conditioned medium, as shown in Figure 3A. In addition, transcripts for these proteins were detectable by RT-PCR in mMSC, WI-38, and IMR-90 cell extracts (Fig. 3B). Densitometric analysis of these data shows that the level of PEDF transcripts in the mMSC samples is at least four times that observed in IMR-90 cells, and approximately twice that in WI-38 fibroblasts (Fig. 3C). PEDF receptor transcripts were also detected in WI-38 and IMR-90 cell extracts (Fig. 3D).

To confirm that PEDF is the major fibroblast chemoattractant in the mMSC secretome, we

compared the chemotactic activity of mMSC conditioned medium that had been immunodepleted of PEDF with the activity of serum-free medium reconstituted with purified PEDF; these experiments are illustrated in Figure 4. Figure 4A shows that removing PEDF with anti-PEDF beads results in a complete loss of IMR-90 and WI-38 chemotaxis, as compared to conditioned medium treated with (negative control) beads cross-linked with non-specific IgG; fibroblast chemoattractive activity is reconstituted by adding PEDF to serum-free medium. The transmigration data in Figure 4B shows that PEDF purified from FBS stimulated chemotaxis of IMR-90 and WI-38 fibroblasts in a dose-dependent fashion, ~1 nM PEDF being sufficient to generate ~50% of the maximum response; the PEDF concentration used in this experiments was chosen to approximate levels used in previous studies [Dawson et al., 1999; Filleur et al., 2005]. Similar fibroblast chemotactic activity was observed with PEDF purified from human plasma (data not shown). From these depletion and reconstitution experiments, we conclude that PEDF is the primary chemoattractant for fibroblasts in the mMSC secretome.

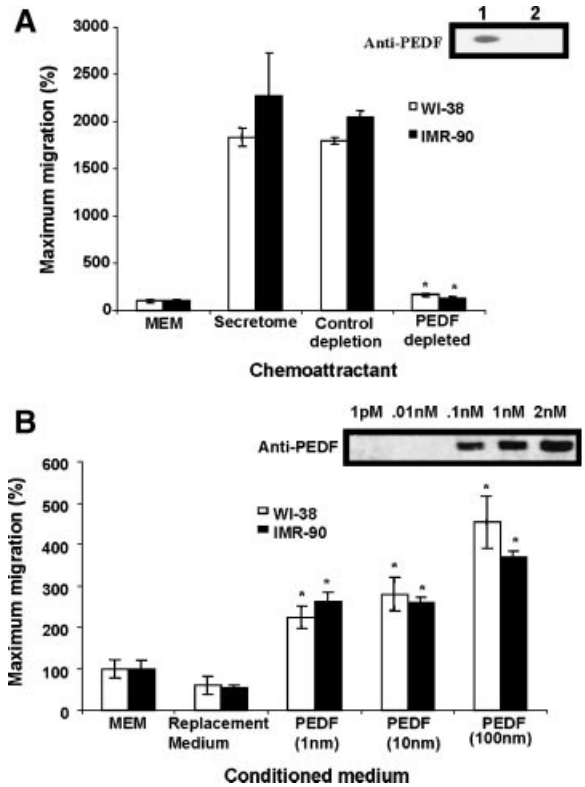

Fig. 4. Effect of PEDF immunodepletion of the mMSC secretome, and reconstitution with PEDF, on fibroblast chemotactic activity. **A**: Rabbit anti-PEDF or purified rabbit IgG were cross-linked to ProteinA/G agarose beads (Pierce Biotechnology) were added to the mMSC secretome, and processed as described in Materials and Methods Section. The resulting supernatants were used in fibroblast chemotactic assays. The presence of PEDF bound to the cross-linked antibody beads was confirmed by Immunoblot analysis (*insert*: **lane 1**, anti-PEDF cross-linked beads; **lane 2**, control beads with purified IgG). Data are the mean ± SD from three independent assays. *Significantly different from secretome and control depletion, $P < 0.05$. **B**: PEDF purified from fetal bovine serum by collagen-Sepharose affinity chromatography (9) was added to protein-free MEM at the indicated concentrations. Similar results were obtained using PEDF purified from human plasma (data not shown). (The *insert* shows PEDF purified from fetal bovine serum at different concentrations, using immunoblot analysis). ''Replacement medium'' refers to MEM supplemented with Serum Replacement 1 (Sigma catalog #S0638). Data are the mean ± SD from three independent assays. *Significantly different from plain MEM control and replacement medium, $P < 0.05$.

DISCUSSION

In this work, we enriched a plastic-adherent mouse bone marrow cell line exhibiting surface markers characteristic of multipotent mesenchymal stem cells. We showed that conditioned medium from this mMSC is chemotactic for two human fibroblast cell lines, WI-38 and IMR-90. PEDF, a ~50-kDa glycoprotein member of the serpin gene family, was identified as the major chemotactic factor in the secretome. Eighteen other secreted proteins were also identified in the mMSC secretome.

PEDF exhibits several biological activities, including promoting neuronal cell survival, anti-angiogenesis, and inhibition of tumor metastasis [Gettins et al., 2002; Tombran-Tink and Barnstable, 2003]. It is synthesized by retinal pigment epithelial cells and secreted into the retinal inter-photoreceptor matrix, where it influences development and differentiation of the neuroretina [Steele et al., 1993]. More recently, Ramirez-Castillejo et al. [2006] identified PEDF as critical for communication between vascular and neural cells in an adult neural stem cell niche, the subventricular zone. PEDF is also expressed by astrocytes, another key cellular element in the adult neural niche [Alvarez-Buylla and Lim, 2004], but is not detected in the conditioned medium, possibly because of its sequestration to the cell surface or extracellular matrix, which may have prevented its release into the medium [Ramirez-Castillejo et al., 2006]. PEDF is released when cells become confluent, and suppresses cell cycle

progression [Pignolo et al., 2003]. This may represent a significant mechanism to halt cell cycle traverse, to commence terminal differentiation programs.

In our study, PEDF stimulates fibroblast migration. In contrast, other investigators have reported that PEDF inhibits growth factor-stimulated migration of endothelial cells [Dawson et al., 1999; Filleur et al., 2005], but by itself has no effect on endothelial cell migration [Dawson et al., 1999]. This inhibitory effect of PEDF on growth factor-stimulated endothelial cell migration is consistent with its known role in preventing angiogenesis [Filleur et al., 2005]. This differential effect of PEDF on cellular migration of these two cell types suggests that this protein may play a role in regulating the cellular profile at an injury site. It is reasonable to expect that cellular requirements may differ between early and late post-injury stages. Factors such as PEDF, secreted by resident stem cells, may stimulate migration of cells needed early in regeneration, such as fibroblasts for scaffold building, while inhibiting migration of cells such as endothelial cells, needed for capillary formation at subsequent stages of healing. In this way, PEDF may play a role in defining a temporal strategy for allocation of tissue resources during tissue regeneration.

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