



# Endothelial-monocyte-activating polypeptide II induces migration of endothelial progenitor cells via the chemokine receptor CXCR3

Yonghao Hou<sup>a</sup>, P. Artur Plett<sup>b</sup>,  
David A. Ingram<sup>c,d</sup>, Gangaraju Rajashekhar<sup>a</sup>, Christie M. Orschell<sup>b</sup>,  
Mervin C. Yoder<sup>c,d</sup>, Keith L. March<sup>a</sup>, and Matthias Clauss<sup>a</sup>

<sup>a</sup>Department of Cellular and Integrative Physiology and Indiana Center for Vascular Biology and Medicine; <sup>b</sup>Department of Pediatrics, Herman B. Wells Center for Pediatric Research; <sup>c</sup>Division of Hematology/Oncology, Department of Medicine; <sup>d</sup>Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Ind., USA

**Objective.** Recruitment of endothelial progenitor cells to the sites of ischemia has recently been suggested as a mechanism of tissue repair. Here we address the hypothesis that the hypoxia-inducible full-length endothelial-monocyte-activating polypeptide II (EMAP II) provides a mechanism to recruit late outgrowth highly proliferating endothelial progenitor cells (EPCs).

**Materials and Methods.** We tested in a transwell migration assay EMAP II for its ability to induce migration of EPCs. Furthermore, we measured changes in cellular calcium levels in EPC to assess the ability of EMAP II to induce intracellular signaling. Finally, we employed neutralizing antibodies and binding competition studies in order to identify the receptor mediating these activities of EMAP II in EPCs.

**Results.** EMAP II elicits dose-dependent migration and intracellular calcium mobilization in EPCs. Functional blocking and binding studies with radiolabeled interferon- $\gamma$ -induced protein (IP-10) indicate that EMAP II employs the CXCR3 receptor for these activities in EPCs. Indeed, EMAP II-induced migration of EPCs can be abolished by prior treatment of cells with anti-CXCR3 antibodies or with IP-10.

**Conclusions.** These data suggests a novel function for EMAP II and a hitherto undescribed role of the CXCR3 chemokine receptor in EPC recruitment. © 2006 International Society for Experimental Hematology. Published by Elsevier Inc.

Recently, the concept that tissue repair processes involve the role of adult progenitor cells has attracted much attention. For instance, in cardiovascular ischemia, mesenchymal, or circulating endothelial progenitor cells contribute to repair processes within the vasculature and even within cardiomyocytes [1–3]. Circulating endothelial progenitor cells (EPCs) have been identified as bone marrow-derived cells, which appear to best be characterized by the expression of CD34, the vascular endothelial growth factor receptor (VEGFR)-2, and CD133 [2]. However, some controversy about the nature of circulating endothelial progenitor cells emerged, which was reflected by description of different isolation and cultivation protocols. According to

one protocol, early outgrowth EPCs are described as cells of low proliferative potential that also express markers for monocytic lineages [4,5]. According to another protocol, late outgrowth EPCs exclusively express endothelial cell markers and demonstrate higher proliferative capacities [5–7]. In the following, the term *EPC* will be used for cells isolated and characterized according to the latter description of late outgrowth and highly proliferative circulating endothelial progenitor cells, which are clearly distinguished from peripheral blood monocytes [5–8].

Although the concept that EPCs can participate in tissue repair is by now widely accepted, the mechanism underlying the recruitment of these cells are only partially explored. One common element is the presence of a hypoxic stimulus. Accordingly, proteins that are directly regulated by hypoxia-inducible factor-1 (HIF-1), such as the vascular endothelial growth factor (VEGF) and the

Offprint requests to: Matthias Clauss, Ph.D., Indiana Center of Vascular Biology and Medicine, Indiana University School of Medicine, 975 West Walnut Street IB464, Indianapolis, IN 46202; E-mail: [mclauss@iupui.edu](mailto:mclauss@iupui.edu)

chemokine stromal cell-derived factor-1 (SDF-1), have been implied in processes of progenitor cell mobilization and recruitment to sites of hypoxia. VEGF and homologues binding to either VEGFR-1 or VEGFR-2 have been demonstrated to mobilize monocytes and EPC [9–11]. However, a cautious note about the use of VEGFR-1 binding VEGF family members was raised based on their involvement in arthritis and atherosclerosis [12]. In addition to VEGF, another hypoxia inducible protein, SDF-1 and its receptor CXCR4, had been identified as critical mediators for the ischemia-specific recruitment of circulating progenitor cells [13]. Recent evidence infers a contribution of smooth muscle cell (SMC) progenitors and SDF-1 to neointima formation after arterial injury [14]. Inhibition of plaque area and SMC content in apolipoprotein E-deficient mice repopulated with LacZ<sup>+</sup> or CXCR4<sup>-/-</sup> bone marrow (BM) or lentiviral transfer of an antagonist reveals a crucial involvement of local SDF-1 and its receptor CXCR4 in neointimal hyperplasia via recruitment of BM-derived SMC progenitors. Therefore, analysis of recruiting mechanisms for a wide range of circulating progenitor cells is helpful to evaluate the range of possible therapeutic targets and to address possible side effects.

In an attempt to identify more candidate molecules, potentially involved in the progenitor cell homing processes induced by ischemia, we investigated a protein known to be regulated by ischemia, the endothelial-monocyte-activating polypeptide II (EMAP II). The full-length EMAP II, which is also known as p43 [15,16], has previously been reported to be induced and released by apoptosis, cellular stress, and hypoxia [17–20]. Furthermore, full-length EMAP II, can attract and activate monocytes [16,21]. In this article, the abbreviation EMAP II will be exclusively used for the full-length EMAP II in order to distinguish it from its C-terminal cleavage product, mature EMAP II [17]. Although EMAP II is not considered to belong to the family of chemokines, it displays sequence and structural homologies to chemokines [22–24]. In this study, we demonstrate that the full-length EMAP II can recruit EPCs and we provide evidence for the hypothesis that these activities are mediated by the chemokine receptor CXCR3 as an EMAP II binding and signaling receptor.

## Materials and methods

### Reagents

VEGF was obtained from Sigma (St. Louis, MO, USA). Human recombinant interferon- $\gamma$ -induced protein (IP-10; cat. no. 266 IP) and monoclonal antibodies to anti-CXCR3 (cat. no. MAB160) were obtained from R&D Systems, Minneapolis, MN, USA).

### EMAP II production

The full-length form of EMAP II coding region was cloned into pPICZ A vector (Easy Select, Pichia Expression Kit;

Invitrogen, Carlsbad, CA, USA), which contains co-mined His- and Myc-tags at the C terminal. Expression of EMAP II was carried out according to manufacturer's instructions and confirmed by Western blot analysis using rat polyclonal antibodies toward EMAP II SA2846. Purification of EMAP II was performed by affinity chromatography using a Ni Sepharose 6 Fast Flow column (Amersham Biosciences, Arlington Heights, IL, USA). Protein concentration was determined using bicinchoninic acid protein assay kit from Pierce (Rockford, IL, USA).

### CD34<sup>+</sup> cell source

Human BM aspirates were obtained from normal adult volunteers after receiving informed consent according to guidelines established by the Human Investigation Committee of the Indiana University School of Medicine. Cells were isolated as described previously [25]. Briefly, low-density BM cells were separated over Ficoll-Hypaque (Pharmacia, Piscataway, NJ, USA). CD34<sup>+</sup> isolation was performed using magnetic cell separation columns (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) and antibodies recognizing the CD34 epitope QBEND/10 according to the manufacturer's procedure. Purity of CD34<sup>+</sup>-selected samples ranged from 70% to 80%.

### Isolation and cultivation of endothelial progenitor cells

Highly proliferative late outgrowth EPCs clones were isolated from human umbilical cord blood as described previously [7,8,26]. Briefly, mononuclear cells (MNCs) from umbilical cords of healthy term infants were isolated using gradient centrifugation over Ficoll. Isolated cells were cultivated with endothelial growth medium-2 (EGM-2; Cambrex, Walkersville, MD, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 2% penicillin/streptomycin (Invitrogen, Grand Island, NY, USA), and 0.25 g/mL amphotericin B (Invitrogen) (complete EGM-2) and highly proliferative clones were further expanded. Cells were maintained in culture at EGM-2 complete medium from Cambrex plus 10% FBS and used at passage number <10.

### Transwell migration assays

Migration of endothelial progenitor cells to EMAP II was assessed as described previously with minor modifications [21]. Briefly, EPCs were placed in 100  $\mu$ L into the upper chamber of 6.5-mm diameter, 5.0- $\mu$ m pore size transwell according to the instruction of the company (Costar, #3421, Cambridge, MA, USA). Collagen-coated filters were employed to enhance adhesion. Because almost all cells stay at the lower side of the membrane after migration, quantification can be performed by simply counting these cells. We used EMAP II or other factors of interest were added to the lower chambers. In some cases cells were pre-treated with either 10  $\mu$ g/mL CXCR3 antibody, or 1  $\mu$ g/mL: IP-10 (agonist of CXCR3), for 20 minutes before put into migration assay. For the CXCR3 antibody studies, equal

amount of antibodies were also added to the lower wells. The assays were conducted over a 4-hour incubation period at 37°C in a CO<sub>2</sub> (7.5%) equilibrated incubator. After migration, nonmigrating cells were removed by cotton-tipped applicators from the upper side of the transwells and membranes of transwell filters were fixed and stained by Hema 3 STAT PACK protocol (Fisher Scientific, no. 123-869, Hampton, NH, USA). Cells adhered at the lower side of the membranes were counted under the microscopy in five to six high-power fields/well and the average/standard error of the average was determined. For assessing migration of CD34<sup>+</sup> bone marrow-derived cells, cells were harvested from the bottom of a transwell and quantified by flow cytometry as described previously [25]. Briefly, 0.8–1.0 × 10<sup>5</sup> cells were allowed to migrate for 3 to 4 hours across transwells to bottom chambers containing EMAP II in the absence or presence of neutralizing antibodies or 100 ng/mL SDF-1 as a positive control or media only. Migrated cells were collected and stained with anti-CD34-PE-Cy5. Migration of CD34<sup>+</sup> cells was quantified by adding a fixed number of carboxy-fluorescein diacetate, succinimide ester (CFSE)-labeled cells to migrated cells and comparing to a standard curve prepared with known amounts of unlabeled cells and CFSE-labeled cells. CFSE-labeled cells were added just prior to acquisition to avoid CFSE contamination of migrated cells.

#### Intracellular calcium flux measurements

Endothelial progenitor cells were seeded into 96-well plate (black, clear bottom; NUNC, Rochester, NY, USA) at 40,000/well the day before the assay to form monolayer. Cells were loaded with FLPR Calcium 3 Assay kit according to manufacturer's instruction. After 40 minutes to 1 hour at 37°C incubation cells were put into assay plate chamber of FlexStation II (FLPR Calcium 3 Assay kit; Molecular Devices, Sunnyvale, CA USA) and compounds of interest were added into assay plate by automatic transfer. Calcium flux shown as relative fluorescence units in 5- to 20-second intervals was recorded up to 200 seconds after addition of compounds and quantified using the software provided by Molecular Devices (SoftMax Pro4.6).

#### Binding competition assays

Binding assays were performed as described previously [27]. In brief, cells were plated in 24-well culture plates and incubated overnight in standard medium. For the binding assay, cells were washed twice with washing buffer (0.5 M NaCl, 50 mM Hepes, 1 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, and 1% bovine serum albumin, pH 7.2), once with binding buffer (washing buffer without NaCl), and then incubated in duplicates with a constant concentration (18 pM) of <sup>125</sup>I-labeled IP-10 (Amersham Biosciences) in the presence of increasing concentrations of unlabeled EMAP II. Incubations took place in 200 μL binding buffer. After incubation at room temperature for 2 hours, binding buffer was aspi-

rated and cells were washed once in PBS, lysed in 0.5 mL 1 N NaOH and radioactivity was determined using a gamma counter.

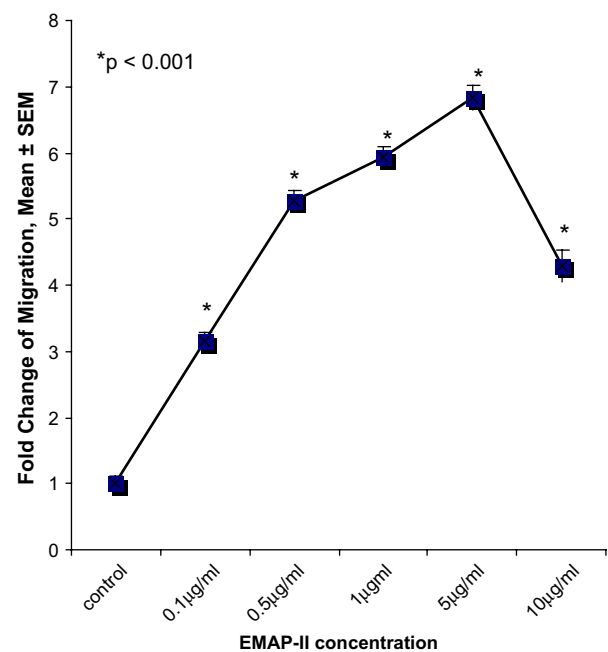
#### Statistical analysis

A paired *t*-test was utilized to establish statistically significant differences between treatment groups. Where applicable, mean ± SEM of multiple measurements is reported, as indicated.

## Results

#### EMAP II induces migration of EPCs

In order to test the potential ability of EMAP II to recruit late outgrowth EPCs, we tested the ability of EPCs to migrate in response to increasing EMAP II concentrations. As shown in Figure 1A, endothelial progenitor cells migrate toward EMAP II. The response to EMAP II to EPC was found to be concentration-dependent with maximal activities between 1000 and 5000 ng/mL and to decline at 10,000 ng/mL (Fig. 1A). In addition, we tested EMAP II to recruit human bone marrow-derived CD34<sup>+</sup> stem/progenitor cells purified from human donors. Testing bone marrow derived CD34<sup>+</sup> cells, we observed a small but statistically significant increase in migration at an EMAP II



**Figure 1.** Endothelial-monocyte-activating polypeptide II (EMAP II) elicits migration of progenitor cells. (A) Dose response of EMAP II with endothelial progenitor cells (EPCs). Migration of EPCs was expressed as fold change increase over spontaneous medium control (arbitrarily set as 1) by counting migrated cells adherent to undersurface of transwell membrane stained by hematoxylin. All values are significantly increased over control ( $p < 0.001$ ). Shown are mean ± SEM of one representative experiment out of two.

concentration of 100 ng/mL over spontaneous (0.9–2.0%,  $n = 3-5$ ,  $p < 0.05$ ). Together, these data demonstrate that EMAP II can recruit circulating endothelial progenitor cells. In addition, we found some preliminary evidence for the hypothesis that EMAP II can also induce migration of bone marrow-derived CD34<sup>+</sup> progenitor cells.

#### Evidence for a relation of EMAP II to the family of chemokines

The chemotactic activity, which was demonstrated by checkerboard analysis for monocytes in a previous report [22] and in this study for EPCs (data not shown) suggests that EMAP II may be related to the family of chemokines. This proposition is supported by the 50% sequence homology of EMAP II to interleukin (IL)-8, which occurs within a 10 amino acid long region occurs right at the definition site for chemokines (underlined amino acids in the one letter code, Fig. 2A). In addition, there is also a considerable

40% overlap with platelet factor 4 (PF4) in this region (amino acids in bold, Fig. 2A), which defines CXC-chemokines and carries an ELR (IL-8)/DLQ (PF4) motif involved both in myeloid progenitor proliferation and angiogenesis control [28,29]. Based on the hypothesis that EMAP II is related to chemokines, we tested the possibility that it binds to and signals through a chemokine receptor. This hypothesis is supported by the finding that EMAP II-induced migratory activity to EPCs can be abolished by pertussis toxin, which blocks a G protein coupled signal transduction pathway as a common characteristic feature for the chemokine family of receptors (Fig. 2B).

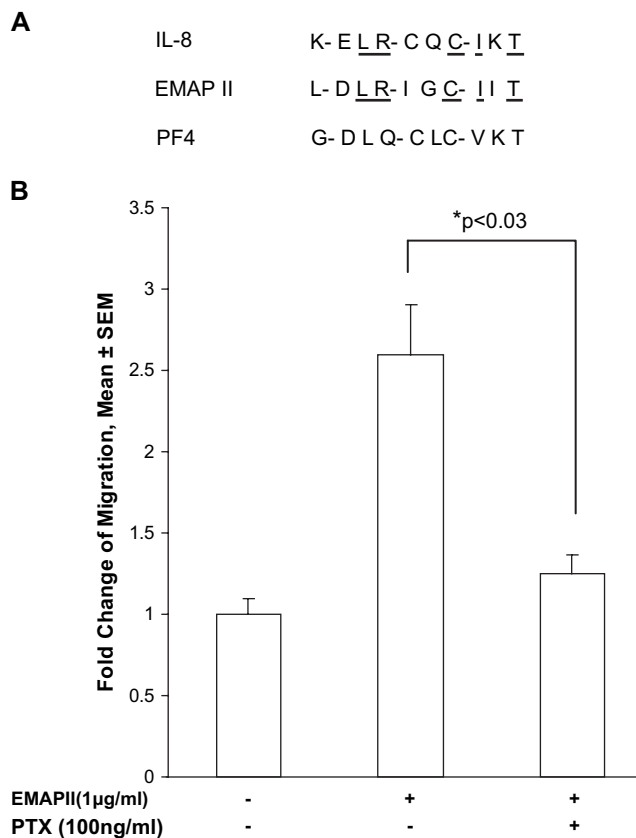
#### Identification of a signaling receptor for EMAP II

Having shown that EMAP II is dependent on G protein coupled signal transduction (Fig. 2B), we assessed the ability of EMAP II to elicit intracellular calcium signaling in EPCs, which is another hallmark in chemokine activation and a generally employed tool for testing chemokine receptor signaling. As shown in Figure 3A, EMAP II causes biphasic dose response in intracellular calcium fluxes in EPCs.

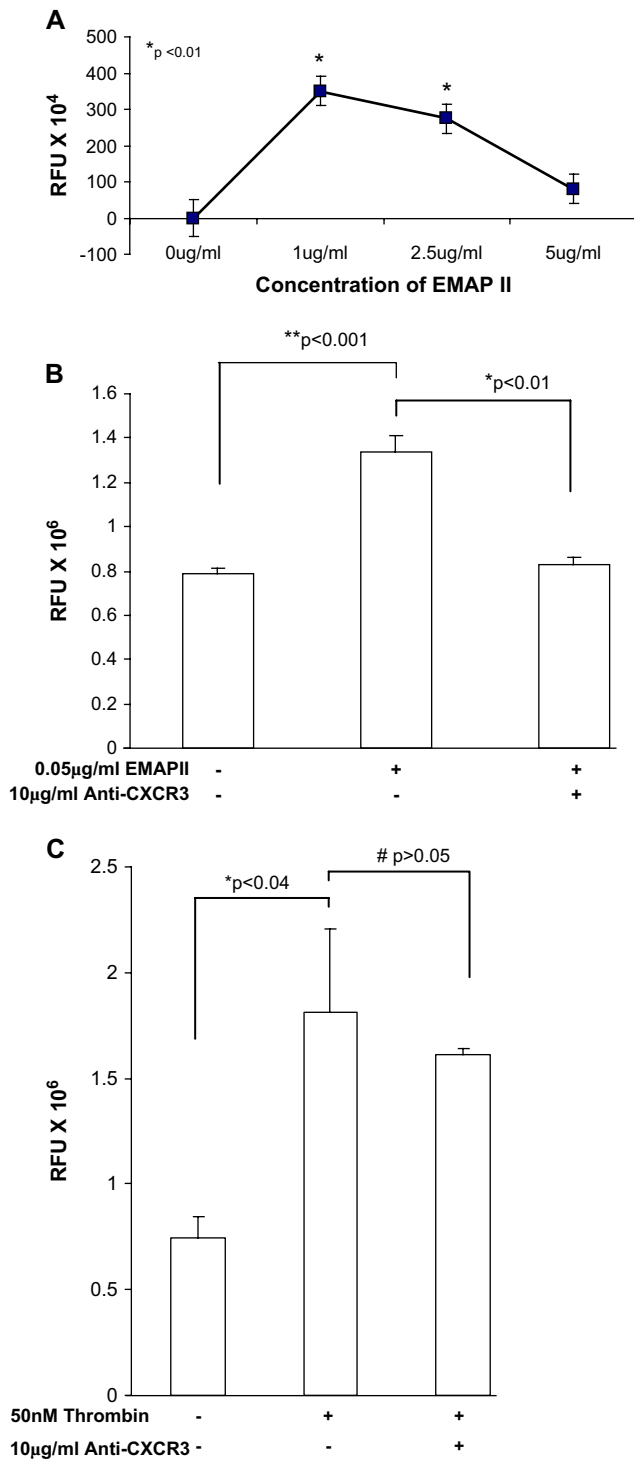
Based on the known antiangiogenic activities of EMAP II, we decided to test for CXCR3 chemokine receptor, which has been described in endothelial cells to mediate the anti-angiogenic activity of PF-4, IP-10, and ITAC [30]. Therefore, we tested a neutralizing anti-CXCR3 antibody for its ability to reduce EMAP II activity on calcium signal induction (Fig. 3B). In these experiments, EPCs were preincubated with 10  $\mu$ g/mL anti-CXCR3 antibodies during the loading time and then subjected to stimulation with 0.05  $\mu$ g/mL EMAP II or 50 nM thrombin. Complete inhibition of calcium flux was observed in EMAP II but not in thrombin-stimulated EPCs (Fig. 3B and C). These data demonstrate that CXCR3 antibodies inhibit EMAP II-induced signal transduction based on intracellular calcium mobilization.

#### EMAP II competes with the CXCR3 receptor ligand IP-10 in binding studies

To further address the hypothesis that EMAP II-induced signaling involves the CXCR3 receptor, we performed binding competition studies with the cognate CXCR3 ligand, IP-10. As shown in Figure 4, EMAP II is able to competitively displace <sup>125</sup>I labeled IP-10 on endothelial progenitor cells (triangles). To test the possibility that EMAP II binds to a splice variant of CXCR3, as it was described for PF4 binding to CXCR3B we used an epithelial cell line transfected with CXCR3 (CXCR3A) and CXCR3B [27]. Whereas unspecific binding of <sup>125</sup>I labeled IP-10 to mock transfected epithelial cells was not competed at all concentrations of EMAP II being used (mock, open circles), EMAP II competed for IP-10 binding to CXCR3 (CXCR3, diamonds) and CXCR3B (CXCR3B, squares) transfected epithelial cells at similar concentrations as



**Figure 2.** Evidence for endothelial-monocyte-activating polypeptide II (EMAP II) as a chemokine-related polypeptide. (A) Homology stretch of the EMAP II protein sequence within a chemokine-defining sequence with interleukin-8 (IL-8) and platelet factor 4 (PF4). (B) Endothelial progenitor cells (EPCs) migration toward EMAP II is pertussis toxin (PTX)-sensitive. Endothelial progenitor cells were pretreated with 100 ng/mL PTX for 20 minutes before being subjected to 1  $\mu$ g/mL EMAP II in lower chamber of transwell. PTX-pretreated cells showed reduced migration close to the level of medium control. Shown are mean  $\pm$  SEM of one experiment.



**Figure 3.** Endothelial-monocyte-activating polypeptide II (EMAP II) induces intracellular calcium changes in endothelial progenitor cells (EPCs). Shown are intracellular calcium changes in FURA-2 loaded EPCs and online measurement using FLPR technology. Concentration dependence of calcium mobilization in response to EMAP II (A). Neutralizing anti-CXCR3 antibodies (10 µg/mL) significantly blocked intracellular calcium signals induced by EMAP-II (B) but not induced by thrombin (C). Shown are mean ± SEM of one representative experiment out two (B,C) or three (A), respectively.

observed with EPCs (EPCs, triangles). These data indicate that EMAP II is able to bind to CXCR3 and its splice variant CXCR3B.

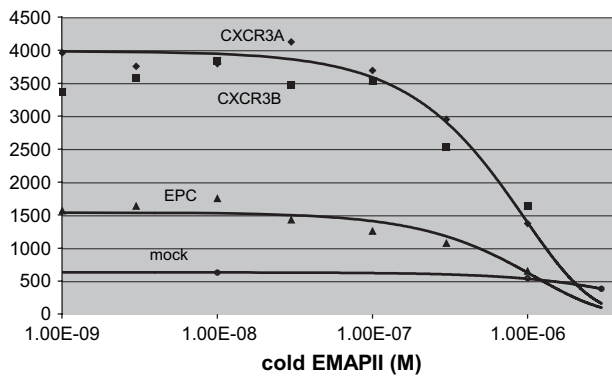
*Involvement of the CXCR3 receptor in EMAP II-induced migration of EPCs*

To further extend our finding that CXCR3 inhibition results in blockade of EMAP II-induced intracellular signaling, we tested whether EMAP II-induced migration was reduced by using neutralizing anti-CXCR3 antibodies (10 µg/mL). As shown in Figure 5A, migration of EPCs in response to EMAP II was blocked by anti-CXCR3 antibodies. In addition, pretreatment of EPCs with an agonist of CXCR3, IP-10, abolishes EMAP II-mediated recruitment of EPCs to levels comparable to spontaneous migration (Fig. 5B). Taken together, we conclude that endothelial progenitor cells use CXCR3 as the receptor to respond to EMAP II.

**Discussion**

Our study demonstrates for the first time that EMAP II can attract endothelial progenitor cells. This suggests that EMAP can be involved in the regulators of angiogenesis and tissue repair in ischemic disease. Because monocytes have been demonstrated to be involved in these activities and previous reports have shown that EMAP II is a chemoattractant for monocytes [5–7,16,17,21,31], we have purposely chosen a protocol leading to EPCs, which are defined as late outgrowth cells and do not express monocytic markers [5–8]. In addition, we have observed that EMAP II can also induce the migration of a very small proportion of bone marrow-derived CD34<sup>+</sup> cells, and subsequent fluorescein-activated cell sorting analysis of migrated cells showed enrichment of CD133<sup>+</sup> cells (data not shown). Of note, CD133 is a plasma membrane marker for early progenitor cells, including hematopoietic and endothelial progenitor cells [2,32].

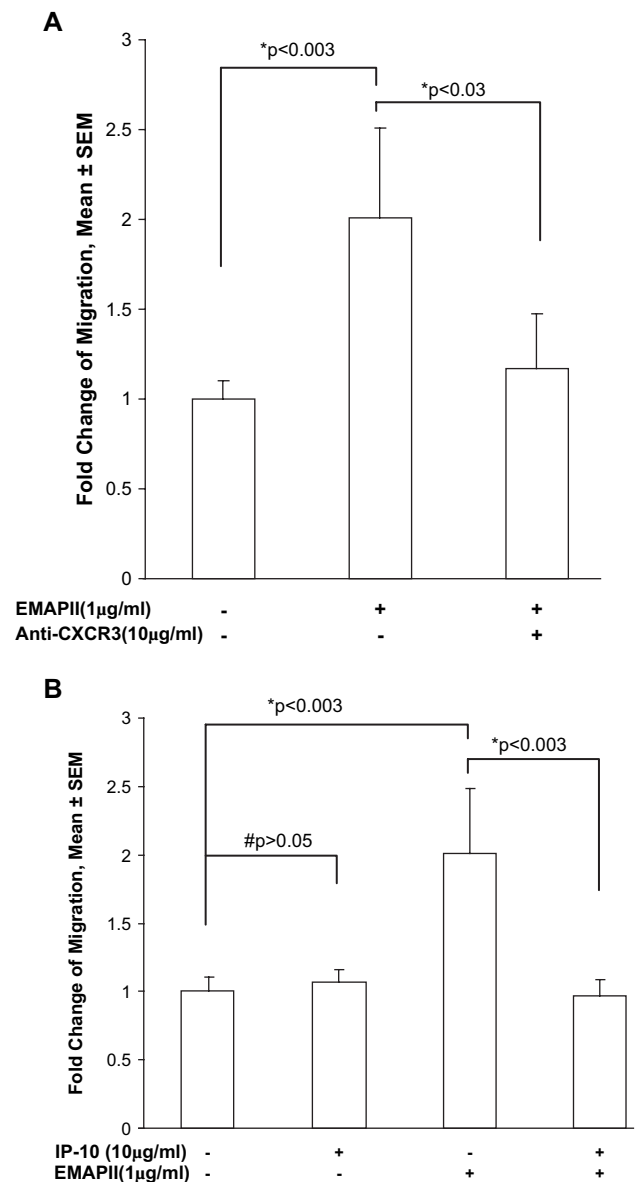
In order to obtain further insights into the underlying mechanism leading to migration of cells, we tested signaling events and signaling inhibitors. Our finding that EPC migration induced by EMAP II was abolished by treating cells with pertussis toxin B indicates that EMAP II employs a G-protein coupled receptor as it is characteristic for chemokines. This is in line with a selective EMAP II sequence homologies to IL-8 and PF4 within a chemokine consensus sequence (Fig. 2). In addition, EMAP II induces intracellular calcium in EPC, which is another hallmark in chemokine receptor activation [33]. Neutralizing antibodies against CXCR3 blocked both EMAP-induced intracellular calcium increase (Fig. 3B) and migration of endothelial progenitors (Fig. 5A). Furthermore, the CXCR3 ligand, which cannot cause EPC migration by itself, was able to block EPCs to respond to EMAP II (Fig. 4). This can be explained either by receptor desensitization in favor for the hypothesis that EMAP II and IP-10 share the same receptor



**Figure 4.** Competition studies of cold endothelial-monocyte-activating polypeptide II (EMAP II) with  $^{125}$ -iodine-labeled interferon- $\gamma$  induced protein (IP-10) for CXCR3 binding. Endothelial progenitor cells (EPCs) were incubated for 2 hours with 18 pM  $^{125}$ -iodine-labeled IP-10 in the presence of increasing concentrations of unlabelled pro EMAP II as described in Materials and Methods. Of note, IP-10 binding in EPCs (EPC) is weaker in comparison to CXCR3 and CXCR3B transfected immortalized cells but still significant higher than in mock transfected control cells. Shown is one representative experiment out of two.

although a general inhibitory effect of IP-10 on motility as shown for VEGF-induced migration cannot be excluded [34]. Finally, EMAP II was able to compete with IP-10 for binding to EPCs as well as to CXCR3 transfected cell lines. Although binding competition studies revealed less affine binding to CXCR3 as it was reported for other CXCR3 ligands such as Mig, PF-4 or ITAC [35] migration induction by EMAP II also occurs at higher concentration as reported previously for these and other chemokines.

Although CXCR3 has been best explored for its role in lymphocytes such as activated T cells, recently, several reports indicated CXCR3 expression in endothelial cells [27,34,35]. Of note, in endothelial cells, a splice variant of CXCR3, CXCR3B, was demonstrated to mediate the inhibitory effect on endothelial proliferation of antiangiogenic chemokines such as PF4 and IP-10 [27]. In this context, EMAP II had been also described to display antiangiogenic activity in growing endothelium [36], which may be related to restriction of CXCR3 expression to proliferating endothelium [30]. Alternatively, the interaction of EMAP II with its extracellular binding protein,  $\alpha$ -adenosine triphosphate (ATP) synthase, in serum-starved endothelial cells was demonstrated to provide an antiproliferative stimulus [37]. Importantly, in an *in vivo* angiogenesis assay, the chicken chorioallantois membrane assay, EMAP II displayed a biphasic dose response: at lower concentrations it displayed proangiogenic and at higher concentrations antiangiogenic properties [38]. Although angiogenic effects of EMAP II are not the focus of our study, it could be hypothesized that at low concentrations EMAP II application favors recruitment of EPCs to the angiogenic vessels, whereas at higher concentrations blockade of  $\alpha$ -ATP synthase



**Figure 5.** Inhibition of endothelial-monocyte-activating polypeptide II (EMAP II)-induced endothelial progenitor cells (EPCs) migration. Cells were pretreated with either antibodies or interferon- $\gamma$ -induced protein (IP-10) and added to the upper wells of the migration chamber (A). Pretreatment of EPC with neutralizing anti-CXCR3 antibodies (10  $\mu$ g/mL) inhibits EMAP II (1  $\mu$ g/mL)-induced migration. (B) Pretreatment of EPCs with IP-10 (1  $\mu$ g/mL) abolishes EMAP II-induced migration. Shown are mean  $\pm$  SEM, of one representative experiment out of three.

becomes important resulting in inhibition of endothelial cell proliferation.

In conclusion, our finding that the hypoxia-inducible EMAP II can attract EPCs suggests that it may be involved in homing of EPCs and thus in angiogenesis and tissue repair. A receptor shared by many angiostatic chemokines, CXCR3, possibly its splice variant CXCR3B, is utilized by EMAP II to elicit its signal cascade, including free calcium increase and migration in endothelial progenitor cells.

Additional studies addressing this EMAP II CXCR3 interaction in vivo will further address the biomedical importance of our in vitro study.

### Acknowledgments

We are grateful to Peichuan Sun and Liyun Cao (Department of Cellular and Integrative Physiology) and Rita Mitnacht-Kraus (W. G. Kerckhoff/Max-Planck-Institute, Bad Nauheim, Germany) for their excellent technical assistance. We would also like to thank Paola Romagnani (University of Florence, Firenze, Italy) for providing us with CXCR3 and CXCR3B transfected epithelial cells and for her valuable advice. This study was supported by the Indiana Center for Vascular Biology and Medicine and the Cryptic Masons Medical Research Foundation. D. I. and M. Y. are founding members of EndGenitor Technologies, Inc.

### References

1. Abedin M, Tintut Y, Demer LL. Mesenchymal stem cells and the artery wall. *Circ Res.* 2004;95:671–676.
2. Urbich C, Dimmeler S. Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res.* 2004;95:343–353.
3. Yoon YS, Lee N, Scadova H. Myocardial regeneration with bone-marrow-derived stem cells. *Biol Cell.* 2005;97:253–263.
4. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* 1997;275:964–967.
5. Rehman J, Li J, Orschell CM, March KL. Peripheral blood “endothelial progenitor cells” are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation.* 2003;107:1164–1169.
6. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest.* 2000;105:71–77.
7. Ingram DA, Mead LE, Tanaka H, et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood.* 2004;104:2752–2760.
8. Ingram DA, Caplice NM, Yoder MC. Unresolved questions, changing definitions, and novel paradigms for defining endothelial progenitor cells. *Blood.* 2005;106:1525–1531.
9. Asahara T, Takahashi T, Masuda H, et al. VEGF contributes to post-natal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 1999;18:3964–3972.
10. Heil M, Mitnacht-Krauss R, Issbrucker K, et al. An engineered heparin-binding form of VEGF-E (hbVEGF-E). Biological effects in vitro and mobilization of precursor cells. *Angiogenesis.* 2003;6:201–211.
11. Hattori K, Heissig B, Wu Y, et al. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat Med.* 2002;8:841–849.
12. Luttun A, Tjwa M, Moons L, et al. Revascularization of ischemic tissues by PIGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med.* 2002;8:831–840.
13. Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med.* 2004;10:858–864.
14. Zernecke A, Schober A, Bot I, et al. SDF-1alpha/CXCR4 axis is instrumental in neointimal hyperplasia and recruitment of smooth muscle progenitor cells. *Circ Res.* 2005;96:784–791.
15. Quevillon S, Agou F, Robinson JC, Mirande M. The p43 component of the mammalian multi-synthetase complex is likely to be the precursor of the endothelial monocyte-activating polypeptide II cytokine. *J Biol Chem.* 1997;272:32573–32579.
16. Ko YG, Park H, Kim T, et al. A cofactor of tRNA synthetase, p43, is secreted to up-regulate proinflammatory genes. *J Biol Chem.* 2001;276:23028–23033.
17. Knies UE, Behrendorf HA, Mitchell CA, et al. Regulation of endothelial monocyte-activating polypeptide II release by apoptosis. *Proc Natl Acad Sci U S A.* 1998;95:12322–12327.
18. Murray JC, Symonds P, Ward W, et al. Colorectal cancer cells induce lymphocyte apoptosis by an endothelial monocyte-activating polypeptide-II-dependent mechanism. *J Immunol.* 2004;172:274–281.
19. Barnett G, Jakobsen AM, Tas M, Rice K, Carmichael J, Murray JC. Prostate adenocarcinoma cells release the novel proinflammatory polypeptide EMAP-II in response to stress. *Cancer Res.* 2000;60:2850–2857.
20. Matschurat S, Knies UE, Person V, et al. Regulation of EMAP II by Hypoxia. *Am J Pathol.* 2003;162:93–103.
21. Shalak V, Kaminska M, Mitnacht-Kraus R, Vandenabeele P, Clauss M, Mirande M. The EMAPII cytokine is released from the mammalian multisynthetase complex after cleavage of its p43/proEMAPII component. *J Biol Chem.* 2001;276:23769–23776.
22. Kao J, Ryan J, Brett G, et al. Endothelial monocyte-activating polypeptide II. A novel tumor-derived polypeptide that activates host-response mechanisms. *J Biol Chem.* 1992;267:20239–20247.
23. Kim Y, Shin J, Li R, Cheong C, Kim K, Kim S. A novel anti-tumor cytokine contains an RNA binding motif present in aminoacyl-tRNA synthetases. *J Biol Chem.* 2000;275:27062–27068.
24. Renault L, Kerjan P, Pasqualato S, et al. Structure of the EMAPII domain of human aminoacyl-tRNA synthetase complex reveals evolutionary dimer mimicry. *EMBO J.* 2001;20:570–578.
25. Plett PA, Frankovitz SM, Wolber FM, Abonour R, Orschell-Traycoff CM. Treatment of circulating CD34(+) cells with SDF-1alpha or anti-CXCR4 antibody enhances migration and NOD/SCID repopulating potential. *Exp Hematol.* 2002;30:1061–1069.
26. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood.* 2005;105:2783–2786.
27. Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S. CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends Immunol.* 2004;25:201–209.
28. Daly TJ, LaRosa GJ, Dolich S, Maione TE, Cooper S, Broxmeyer HE. High activity suppression of myeloid progenitor proliferation by chimeric mutants of interleukin 8 and platelet factor 4. *J Biol Chem.* 1995;270:23282–23292.
29. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem.* 1995;270:27348–27357.
30. Romagnani P, Annunziato F, Lasagni L, et al. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J Clin Invest.* 2001;107:53–63.
31. Knies UE, Kroger S, Clauss M. Expression of EMAP II in the developing and adult mouse. *Apoptosis.* 2000;5:141–151.
32. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood.* 1997;90:5002–5012.
33. Loetscher P, Seitz M, Clark Lewis I, Baggiolini M, Moser B. Monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 are major attractants for human CD4+ and CD8+ T lymphocytes. *FASEB J.* 1994;8:1055–1060.
34. Bodnar RJ, Yates CC, Wells A. IP-10 blocks vascular endothelial growth factor-induced endothelial cell motility and tube formation via inhibition of calpain. *Circ Res.* 2006;98:617–625.
35. Lasagni L, Francalanci M, Annunziato F, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med.* 2003;197:1537–1549.

36. Schwarz MA, Kandel J, Brett J, et al. Endothelial-monocyte activating polypeptide II, a novel antitumor cytokine that suppresses primary and metastatic tumor growth and induces apoptosis in growing endothelial cells. *J Exp Med.* 1999;190:341–353.
37. Chang SY, Park SG, Kim S, Kang CY. Interaction of the C-terminal domain of p43 and the alpha subunit of ATP synthase. Its functional implication in endothelial cell proliferation. *J Biol Chem.* 2002;277:8388–8394.
38. Park SG, Kang YS, Ahn YH, et al. Dose-dependent biphasic activity of tRNA synthetase-associating factor, p43, in angiogenesis. *J Biol Chem.* 2002;277:45243–45248.