The FASEB Journal express article 10.1096/fj.04-3282fje. Published online July 11, 2005.

Embryonic endothelial progenitor cells expressing a broad range of proangiogenic and remodeling factors enhance vascularization and tissue recovery in acute and chronic ischemia

Christian Kupatt,[‡] Jan Horstkotte,[‡] Georgios A. Vlastos,* Achim Pfosser,[‡] Corinna Lebherz,[‡] Matthias Semisch,* Mark Thalgott,[‡] Kerstin Büttner,[‡] Christian Browarzyk,[‡] Jörg Mages,[§] Reinhard Hoffmann,[‖] Alexander Deten,[¶] Mathias Lamparter,*[,†] Fabian Müller,*[,‡] Heike Beck,* Hildegard Büning,[#] Peter Boekstegers,[‡] and Antonis K. Hatzopoulos*[,†]

*GSF-Research Center for Environment and Health, Institute for Clinical Molecular Biology and Tumor Genetics, 81377 Munich, Germany; [†]Vanderbilt University Medical Center, Division of Cardiovascular Medicine, Nashville, Tennessee 37232-6300; [‡]Internal Medicine I, Klinikum Großhadern, 81377 Munich; [§]Technical University, Institute for Medical Microbiology, 81675 Munich; [‖]Max-von-Pettenkoffer Institute, Bacteriology Department, 80336 Munich; [¶]Leipzig University, Carl-Ludwig Institute of Physiology, 04103 Leipzig, Germany; and [#]Gene Center, Ludwig-Maximilians-University, 81377 Munich, Germany

Christian Kupatt and Jan Horstkotte contributed equally to this work.

Corresponding author: Antonis K. Hatzopoulos, Vanderbilt University, Department of Medicine, Division of Cardiovascular Medicine, 2220 Pierce Ave, Nashville, TN 37232-6300. E-mail: antonis.hatzopoulos@vanderbilt.edu

ABSTRACT

Clonal embryonic endothelial progenitor cells (eEPCs) isolated from embryonic day 7.5 mice home specifically to hypoxic areas in mouse tumor metastases but spare normal organs and do not form carcinomas. Based on these results, we assessed the potential of eEPCs to enhance vascularization and limit organ dysfunction after ischemia in syngenic and xenotypic organisms. The angiogenic potential of eEPCs was evaluated in chronic ischemic rabbit hindlimbs after regional application by retroinfusion. eEPC treatment improved limb perfusion, paralleled by an increase in capillary density and collateral blood vessel number. Systemic eEPC infusion into mice after ischemic cardiac insult increased postischemic heart output measured by a marked improvement in left ventricle developed pressure and both systolic and diastolic functions. In vitro, eEPCs strongly induced vascular outgrowths from aortic rings. To address the molecular basis of this intrinsic angiogenic potential, we investigated the eEPC transcriptome. Genome-wide Affymetrix GeneChip analysis revealed that the eEPCs express a wealth of secreted factors known to induce angiogenesis, tissue remodeling, and organogenesis that may contribute to the eEPC-mediated beneficial effects. Our findings show that eEPCs induce blood vessel growth and cardioprotection in severe ischemic conditions providing a readily available source to study the mechanisms of neovascularization and tissue recovery.

Key words: myocardial infarction • angiogenesis • endothelium-derived factors

As a result of severely damaged tissue, patients suffering from acute myocardial infarction often develop congestive heart failure that restricts their mobility and gravely reduces their quality of life and life expectancy. Recent evidence indicates that progenitor cells for endothelium enhance vascularization and improve tissue recovery after myocardial infarction (1). However, it is not yet clear how endothelial progenitor cells (EPCs) exert their beneficial effects in animal models (2) and patients (3, 4).

We used embryonic endothelial progenitor cells (eEPCs) as a model system (5) to understand their role in vascular development and evaluate their potential as therapeutic agents in adult pathological conditions. We recently showed that eEPCs home to tumor tissue by binding to E- and P-selectin expressed on tumor endothelium, similar to the recruitment of activated lymphocytes and other blood-borne cells at sites of inflammation (6). In mice with multiple metastatic growths, we observed that eEPCs home preferentially to hypoxic metastases but spare well-vascularized tumors and normal organs (7). This specificity is inversely related to the degree of vascular density in the metastasis and directly related to local levels of hypoxia and VEGF expression.

Based on these findings, we investigated the effect of transplanted eEPCs on neovascularization and tissue rescue in two animal models of vascular disease, namely heart ischemia in mice and limb ischemia in rabbits. Our results show that local administration of the embryonic cells in rabbits or systemic injection in mice leads to a measurable increase in neovascularization and improves tissue recovery. To gain further insight in the mechanisms that influence tissue regeneration and blood vessel growth after eEPC application, we obtained a genome-wide expression profile of eEPCs. Our data show that eEPCs are a rich source of secreted proteins that regulate endothelial cell growth and organ development, suggesting a complex repertoire of inducing agents that modulate angiogenesis, tissue remodeling, and organ regeneration after ischemic insult.

MATERIALS AND METHODS

Animals

C57Bl/6 mice and New Zealand rabbits were purchased from Charles River Germany (Sulzfeld, Germany). Animal care and all experimental procedures were performed in strict accordance to the German and National Institutes of Health animal legislation guidelines and were approved by the local animal care and use committees.

Reagents

All chemicals were purchased from Sigma (Deisenhofen, Germany), except rompun (Bayer, Leverkusen, Germany) and ketamine (Ratiopharm, Ulm, Germany). Contrast agent Solutrast 370 was kindly provided by Byk Gulden (Konstanz, Germany).

Cell culture

Mouse eEPCs were isolated and propagated as described previously (5). For detection after transplantation, eEPCs were fluorescently marked with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Leiden, The Netherlands) as described previously (6, 7). For the mouse model of myocardial infarction, fluorescently labeled eEPCs were also obtained after transfection with a recombinant adeno-associated virus (rAAV) containing the enhanced green fluorescent protein (EGFP) and the hygromycin resistance genes and selection under hygromycin (100 μg/ml), yielding a high transfection efficacy (>90%) and a long expression period (>4 wk). In this case, eEPCs were detected in vivo by a rabbit anti-GFP antibody (Mobitec, Goettingen, Germany, 1:100) and a peroxidase-conjugated secondary antibody (Zymed, San Francisco, CA 1:100).

Rabbit aortic ring assays

Descending rabbit aortas (from 2.0-2.5 kg animals) were rapidly excised, rinsed with Krebs solution (8), cut into 2 mm slices, and embedded in 400 μl Matrigel (Becton-Dickenson, Heidelberg, Germany) chamber slides. bFGF (FGF-2, 1 μg) or 5×10^4 eEPCs (in 100 μl endothelial medium EBMII (Alexy, Munich, Germany) were added 24 h later on top of the polymerized Matrigel preventing direct contact to the aortic rings.

Rabbit hindlimb ischemia model

After intravenous anesthesia with rompun (2 g/kg) and ketamine (50 mg/kg), the right femoral artery was excised (day 0; ref 9). At day 7, animals were reanesthetized, and a baseline angiography of both legs was performed by automated injection of Solutrast 370 contrast agent (2 ml/sec, 4 ml) followed by fluoroscopy using a Ziehm system (Nurnberg, Germany). Angiograms were stored at 25 pictures (frames) per second for frame count analysis. Next, we inserted a catheter (1.22 mm outer diameter) into the anterior tibial vein followed by retroinfusion of sodium chloride 0.9% (10 ml) with or without eEPCs (5×10^6) over 30 min in the presence of a cuff occluding venous outflow proximal to the catheter insertion. Pressure was continuously monitored and maintained at 80 mmHg to allow complete filling of the veins. This way of application allowed for a more efficient and homogenous distribution of eEPCs as compared with intra-arterial or intramuscular application [monitored by injection of technetium 99 (^{99}Tc) labeled cells; not shown].

At day 35, a second fluoroscopy of the ischemic hindlimb was performed in anesthetized animals with motor pump-assisted infusion of contrast agent (2 ml/s), yielding comparative analysis of collateral vessel numbers (10) and frame count scores (9) between day 7 and day 35 (changes are given in % of day 7). Subsequently, we obtained tissue samples from the thigh (m. adductor) and calf muscles (m. gastrognemius, m. tibialis anterior) for capillary/muscle fiber ratio quantification as described previously (9). Isolated muscle tissues were also cryosectioned at different time points for histological examination to monitor the presence and localization of the eEPCs. The endothelium of perfused blood vessels was decorated with fluorescein (FITC)-conjugated Helix pomatia lectin (Sigma) that was injected (1 mg/ml, 1 ml volume) into the descending aorta 5 min before hindlimb tissue isolation. Lymphocyte (α-naphthyl butyrate esterase assay) and granulocyte/monocyte (Naphthol AS-D chloroacetate esterase/α-naphthyl

acetate esterase assay) detection in tissue sections of the ischemic and nonischemic limb was performed according to the manufacturer's instructions (Sigma).

Mouse model of ischemia/reperfusion

Animals were anesthetized with ketamine (75 mg/kg) and xylazin (15 mg/kg) and ventilated via a Hugo Sachs Harvard Apparatus respirator (Hugstetten, Germany), and body temperature was maintained with a heated blanket. Recruitment of eEPCs was followed with fluorescence microscopy after ligation of the left anterior descending coronary artery for 20 min and eEPC infusion (2×10^5 cells/animal) into the external jugular vein at the onset of reperfusion (for 3 min). After 15 min of reperfusion, hearts (n=5 per group) were flushed with isothermic saline solution (1 min), dissected, and placed on a microscopic stage. Recruited eEPCs were counted at the surface of the left ventricle under a fluorescence microscope (Ploemopak, Leitz, Wetzlar, Germany) using a charge-coupled device camera (COHU 4400, Prospective Measurements, San Diego, CA) under an N_2 filter block (Leitz; ref 11).

For systemic application of eEPCs, lateral sternotomy was performed, the left anterior descending coronary artery was ligated in the proximal third area using a 7-0 suture and a polyethylene tube as described previously (11). This occlusion for 1 h provided a reproducible left ventricle (LV) infarction of 15-18% LV mass. Twenty-four hours later, 300 µl medium (EBMII) with or without 3×10^5 cells was infused into the tail vein. At day 14, a Millar pressure tip catheter was advanced into the LV and LV developed pressure (LVDP), as well as dP/dt_{max} and dP/dt_{min} were measured (n=8 per group). For comparison, GM-CSF was administered intraperitoneally (0. 5 µg/day) for 10 days as described previously (12). GM-CSF administration led to a 1.6-fold increase of mononuclear cells and a 1.4-fold increase of $CD34^+$ cells within the mononuclear population (FACS analysis, data not shown), indicating a 2.2-fold increase of circulating $CD34^+$ cells.

FITC-conjugated Helix pomatia lectin was injected intravenously 5 min before the end of the experiment to mark endothelial cells of perfused vessels (0.5 mg/ml, 250 µl volume).

Statistical methods

Results are means ± SE. Statistical evaluation was done using one-way ANOVA. Whenever a significant effect was obtained with ANOVA, we used multiple comparison tests between the groups with the Student-Newman-Keul's procedure (SPSS statistical program). Differences between groups were considered significant for $P < 0.05$.

Affymetrix gene array analysis

Total eEPC-RNA was isolated using the Trizol reagent (Sigma) and following the manufacturer's instructions. RNA quality was monitored by optical density measurement, gel electrophoresis, and gene expression analysis using RT-PCR (6). Subsequently, 10 µg of total RNA from three independent preparations were used for biotin-labeled cRNA probe synthesis and hybridization of mouse expression array 430 2.0 gene chips according to the Affymetrix standard protocols (Affymetrix, Santa Clara, CA). Gene chips were scanned and analyzed using the Affymetrix GeneChip Operating Software (GCOS 1.0). Each array was checked for general assay quality (3′-5′ ratios for GAPDH and β-actin <1.1, average background noise <75 and

scaling factors ranging between 0.42 and 0.53). Background-corrected and normalized expression measures from probe level data (cel-files) were obtained by using the robust multiarray average function (rma) implemented in Bioconductor's affy-package (http://www.bioconductor.org), and the obtained table with log 2 values was converted into linear values.

RNA and protein analysis

RNA isolation, reverse transcription (RT), and PCR analysis was performed as described previously (6) using the following gene-specific primers:

Bmp6, 5′-ACTACAACGGCAGTGAGTTAAAAAC-3′, 5′-CTCTCACGACCATATTCCTGTACTT-3′;

Ctgf, 5′-AGCAGCTGGGAGAACTGTGTA-3′, 5′-GCAGAAGGTATTGTCATTGGTAACT-3′;

Dkk3, 5′-CGGCTCGGGGGTATTTTGCTGTG-3′, 5′-CGTGCTGGTCTCATTGTGATAGTTGGG-3′;

Fgf2, 5′-TGCCAACCGGTACCTTGCTATGA-3′, 5′-AGGTCCCGTTTTGGATCCGAGTT-3′;

Fst, 5′-ATAAGACAGAACTGAGCAAGGAAGA-3′, 5′-TCCCTACAAGTCTTTTTACATTTGC-3′;

Ihh, 5′-CCATCACTCAGAGGAGTCTTTACAC-3′, 5′-CAGGAAAATAAGCACATCACTGAA-3′;

Kit-L, 5′-GCCTCGAAGCTTGCGGCTTTCCTATTACTGCTACTGC-3′, 5′-CCTCGAGCATGCAGGAGATCTGCGGGAATCCTGTGAC-3′;

Mif, 5′-CCATGCCTATGTTCATCGTG-3′, 5′-GAACAGCGGTGCAGGTAAGTG-3′;

Mmp2, 5′-GAAGGCTGTGTTCTTCGCAGG-3′, 5′-CACGACAGCATCCAGGTTATCAG-3′;

PdgfA, 5′-CGAGCGGCTGGCTCGAAGTCAG-3′, 5′-GGCCAGATCAGGAAGTTGGCCGAT-3′;

tPA, 5′-GTGTGACTTACCGTGGCACCC-3′, 5′-CATCGTGGAGGTCTTGGTTGCC-3′;

Tfpi, 5′-GGATTATGTAAAGCCAGTGAGAGAA-3′, 5′-CACAGTAGAAGCACCGTATAGGAAT-3′;

Tgfβ1, 5′-GAGACGGAATACAGGGCTTTCG-3′, 5′-CGGGTTGTGTTGGTTGTAGAGG-3′;

Timp1, 5′-CCTAGAGACACACCAGAGATACCAT-3′, 5′-CAGCACTATAGGTCTTTGAGAAAGC-3′;

Timp2, 5′-CTGTTAGGGCAGAGCTGGGAATGTGC-3′, 5′-ACTGGTTGGAGGGTGATTCTTAGACC-3′;

Timp3, 5′- CTTTGGCACTCTGGTCTACACTATT-3′, 5′-GCAAGTAGTAGCAGGACTTGATCTT-3′;

VegfA, 5′-GGGTGCACTGGACCCTGGCT-3′, 5′-GAATTCACCGCCTCGGCTTGTC-3′;

Wnt7b, 5′-AGTGGATCTTTTACGTGTTTCTCTG-3′, 5′-CTGGTTGTAGTAGCCTTGCTTCTC-3′.

For protein detection, 2×10^6 cells were lysed in 600 µl 2× Laemmli buffer and 15 µl per lane were analyzed by gel electrophoresis and Western blotting using standard protocols. The anti-Mif, Timp1, Timp2, and PdgfA antibodies were purchased from R&D Systems (Wiesbaden, Germany).

RESULTS

eEPCs improve hindlimb vascularization

To assess the effect of eEPCs on tissue vascularization in a chronic ischemia model, we applied DiI-labeled eEPCs via retroinfusion into the hindlimbs of rabbits 7 days after excision of the femoral artery (set as day 0). Five days after xenotransplantation (day 12), we detected eEPCs engrafted within the affected muscles of the ischemic area (Fig. 1*A*). Injection of FITC-labeled Helix pomatia lectin in the circulation, 5 min before tissue isolation, marked the muscle endothelium and showed that most eEPCs were associated with the vascular system (Fig. 1*B*).

A time course revealed that the mouse cells survived at least 10 days after xenotransplantation in the rabbit, but no significant eEPC numbers could be detected after 14 days (day 21 after excision; Fig. 1*C*). During their presence in the rabbit tissue, the mouse embryonic cells did not cause an acute inflammatory response, as it was documented by comparing lymphocyte and granulocyte/macrophage counts in tissue sections of ischemic, eEPC-treated, and nonischemic limbs (not shown).

However, the transient association of eEPCs with the host blood vessels strongly induced vascular growth within the ischemic tissue. Four weeks after eEPC retroinfusion, the ratio of capillaries to muscle fiber improved in all muscles examined (M. gastrognemius, M. tibialis ant., and M. adductor) as compared with control untreated limbs (Fig. 1*D*). Retroinfusion of eEPCs also led to an increased number of collateral vessels (201±51% as compared between day 7 to day 35 and between treated and control animals; Fig. 1*E*). The increase in capillary and collateral numbers correlated with better perfusion and improved circulation in the ischemic limbs as visualized and measured by fluoroscopy using a contrast agent (9) at day 35 (Fig. 1*F*).

eEPC administration improves heart function after myocardial ischemia-reperfusion in mice

The efficacy of eEPCs to improve limb perfusion in rabbit hindlimbs prompted us to evaluate their effect after myocardial infarction in mice. To this end, we first tested if eEPCs can be specifically retained in heart ischemic tissue. DiI-labeled eEPCs were injected into the external jugular vein at the onset of reperfusion after 20 min of coronary artery ligation. We observed a higher number of adherent cells per microscopic field in ischemic hearts as compared with nonischemic, sham-operated controls (Fig. 2*A*). Our previous work on the tumor homing

mechanisms of eEPCs showed that selectins expressed in endothelium, and their ligands present on the eEPC surface, mediate this process. In support for a similar homing mechanism in ischemic hearts, we find that pretreatment with the selectin antagonist fucoidin blocks eEPC retention in the ischemic areas (Fig. 2*A*).

In a next step, saline solution (300 μl) with or without 3×10^5 eEPCs marked by DiI, or by stable EGFP expression, was infused systemically via the mouse tail vein 24 h after occlusion of the coronary artery for 1 h. The presence of eEPCs found within or adjacent to vascular structures in the postischemic hearts was confirmed 7 days after infusion (Fig. 2*B*). We obtained similar results with eEPCs stably transfected with EGFP. In this approach, eEPCs were detected at day 14 after injection by immunohistochemistry using an anti-EGFP antibody (not shown).

Invasive functional assessment of heart output demonstrated that eEPC treatment improved residual systolic function as measured by an increase of LVDP (Fig. 2*C*) and dP/dt_{max} (Fig. 2*D*) when compared with control mice injected with saline solution. In parallel fashion, eEPC-treatment attenuated the loss of diastolic function observed in untreated animals (measured by dP/dt_{min}, Fig. 2*E*).

Mice injected subcutaneously for 10 days starting 3 days before ischemia-reperfusion with GM-CSF, an agent thought to mobilize adult EPCs, showed a similar improvement and served as a positive control. However, in contrast to the eEPC application 1 day after infarction, GM-CSF provided no significant enhancement of systolic or diastolic function when it was applied during the postischemic period (day 1 to day 7; Fig. 2*C–E*).

eEPCs induce angiogenesis and are a rich source of secreted protein factors

It has been postulated that besides contributing to the newly built blood vessels, one reason of improved vascularization is the ability of EPCs to induce endogenous angiogenesis through secretion of proangiogenic factors (13). Consistent with this notion, we find that eEPCs enhance sprouting in explanted rabbit aortic rings similarly to angiogenesis-inducing agents such as FGF-2 (Fig. 3). This ability, coupled with the close association of eEPCs with host blood vessels, probably induces a strong, localized, angiogenic response after transplantation.

To begin to understand the molecular basis of this induction, as well as the broader effects on tissue recovery due to application of EPCs after ischemic assault, we investigated the full expression profile of secreted proteins by the eEPCs used in these studies. To this end, total RNA was isolated from cultured cells and gene expression was examined using Affymetrix Genechips (45,101 gene sequences). The data were processed to reveal secreted growth factors and related molecules, as well as proteins with enzymatic activities pertinent to tissue remodeling. The entire list of such molecules organized in functional groups is shown in Table 1.

The composition of the list brings forth a number of interesting observations about the ways eEPCs can influence their immediate environment after engraftment in ischemic areas. First, it is evident that eEPCs produce a large variety of angiogenic factors at high levels. This group includes not only well-recognized factors like VEGFA and VEGFB but also molecules with proangiogenic properties whose mode of action is less well understood (e.g., Aamp; ref 14). Also, molecules placed within other functional groups in Table 1, such as SDF-1, Ihh, and MIF,

have been shown to trigger angiogenesis (15–17). It is likely that this panoply of angiogenic factors will stimulate blood vessel formation through a number of distinct signaling pathways.

Second, eEPCs express high levels of molecules like thymosin-β_4, an important mediator of wound healing. In a recent study, thymosin-β_4 application enhanced cardiac repair and heart cell survival after infarction (18). Third, there is a strong representation of developmentally active molecules in the wnt and BMP pathways (one-third of the list). Of note, the characteristic properties of the factors in the wnt group predict a strong blockade of the canonical/β-catenin wnt pathway in favor of the noncanonical/Ca^{2+}-mediated wnt signaling. Active suppression of the canonical and activation of the noncanonical pathway is the initial critical step in cardiogenesis (19), and it could also play a role in heart tissue regeneration after injury. In support of this idea, heart-specific overexpression of a secreted frizzled related protein that blocks wnt molecules from binding to their corresponding receptors reduced infarct size and improved heart recovery (20). Fourth, the high levels of metalloprotease inhibitors produced by the eEPCs suggest a moderation of protease activity after eEPC transplantation. Fifth, the embryonic cells produce few of the typical proinflammatory cytokines (i.e., IL-1, IL-2, IL-6), indicating that their presence will not trigger an acute inflammatory response.

To validate the Affymetrix data, the expression of a selected group of genes was analyzed by RT-PCR using gene-specific primers (Fig. 4*A*). Band intensities showed a good correspondence to the Affymetrix values in the majority of cases, indicating that chip data are representative of gene expression levels. To test if mRNA levels reflect protein amounts, we analyzed protein expression of several markers using Western blot analysis. The data showed protein amounts being present in accordance to mRNA levels, indicating that protein synthesis occurs at robust levels (Fig. 4*B*).

DISCUSSION

Tissue regeneration and stimulation of angiogenesis are important considerations in the treatment of myocardial infarction, stroke, and peripheral vascular disease. Recent experimental evidence showed that progenitor cells for endothelium (21) and other somatic cell types (22) exist in adults and that they might play an important role in neovascularization and tissue repair processes. Specifically, cells with endothelial progenitor properties originating in the bone marrow were found to home to the damaged heart tissue (1, 23, 24). Moreover, application of EPCs for therapeutic purposes in animal models (12, 25, 26) and in clinical trials (3, 4, 27) showed that such treatment can improve vascularization and tissue function. The data presented here indicate that endothelial progenitor cells isolated from mouse embryos at the onset of vasculogenesis at embryonic day 7.5 have a beneficial effect when transplanted in adult ischemic sites. This was true in both settings of acute and chronic ischemia. In our hands, regional cell application in the hindlimb through the vein provided a privileged access to the ischemic site as previously shown for delivery of proteins and for gene therapy (28–30).

It has been postulated that the positive effect attributed to EPCs is a stimulation of angiogenesis by increasing the direct supply of building blocks for new vasculature (1). However, our previous work using mice transplanted with bone marrow from GFP-expressing donors in a stroke model showed limited incorporation of GFP-positive cells in blood vessels within the infarct or in the peri-infarct areas, but an abundance of bone marrow derived cells in close

proximity to blood vessels (31). It is possible that such discrepancies depend on the origins of the cells used, the isolation protocols, their treatment before transplantation, as well as the tissue type and disease model.

In support of this notion, we observed that in tumor metastasis models homing is related to poor tumor vascularization, low perfusion, and high VEGF and hypoxia levels (7). Moreover, the percentage of eEPCs incorporated in tumor vessels was much higher in subcutaneous tumors after coimplantation of eEPCs and tumor cells than in tumors embedded in lung tissue after systemic eEPC application (7). We concluded that participation of eEPCs in vascular structures is evident, but the numbers seen cannot fully account for the effect eEPCs had on tumor growth. In the experiments described in this work, close histological examination also revealed that eEPCs incorporate in vascular structures, but many of them could be seen in close vicinity to blood vessels. Similarly, adult bone marrow derived cells recruited to sites of arteriogenesis in the chronic ischemic hindlimb were not predominantly integrated into the endothelial lining but adjacent to the vessel wall (13, 32). Our data and the results of others raise the possibility that the increased vascularity and better tissue performance might be also due to an indirect effect of the presence of the progenitor cells within the stricken tissue.

To address this issue, we have assembled an almost complete list of expressed genes in eEPCs that encode secreted proteins. The expression profiling data show a rich collection of proteins from a variety of distinct pathways, i.e., cytokines, chemokines, angiogenic factors, and an extensive presence of protease inhibitors that moderate tissue remodeling. Particularly surprising was a prominent contribution of molecules involved in the BMP and wnt signaling pathways (~35% of all proteins classified as growth factors). The importance of such molecules and their receptors for the development of many organs including the heart, as well their role in embryonic angiogenesis, raises the possibility that they contribute to the regeneration of heart tissue. It appears that the collective profile of these proteins tips the balance toward inhibition of the wnt and BMP signaling pathways. A similar conclusion was drawn regarding the gene expression profile of embryonic stem (ES) cells (33). It is tempting to consider that such constellation of inhibitors of the two most prominent pathways that induce body plan, organogenesis and cell specification could shield stem or progenitor cells from premature differentiation. This "stem cell shield" could in turn influence in a positive way the survival and recovery of ischemic or injured tissue.

Such a rich source of developmentally active proteins might be a unique advantage of the embryonic cells. This notion could gain steam when expression profiles of other EPCs become available. Support of the hypothesis that EPCs of embryonic and adult origins might have different expression profiles, and thus are suited for different tasks, comes from a first comparison with the recently published expression profile of cytokines in adult bone marrow stroma derived stem cells (34). In this instant, there is only a limited degree of overlap between these cells and the eEPCs used in our study (4 out of 19 cytokines, i.e., TGFβ, PDGF, VEGF-A, and VEGF-B). It is thus possible that EPCs of different origins have distinct expression profiles and thus are suited for different tasks.

The expression of many secreted factors known to induce angiogenesis through distinct pathways, in combination with production of high levels of cardioprotective molecules like thymosin-β_4, might be a distinct advantage of cell-based therapeutic approaches as compared

with single agent treatments. However, it will be particularly interesting to test in the future if the complex profile observed in the expression studies is necessary for efficient induction of angiogenesis and tissue recovery or one could substitute cell transplantation with representative members from various groups. It is encouraging that although the protein repertoire is complex, it involves a relatively small number of individual pathways. This raises hopes that in the future cell-based therapies that might lead to unwanted side effects (tumorogenicity of stem cells, persistence in tissues beyond the required time, uncontrollable long-term effects in unsuspected areas within the body) could be substituted by cocktails of proteins that will perform multiple tasks simultaneously or by specifically engineered cells with properties tailored to the particular disease.

Of note, we did not observe any adverse effect due to the xenotransplantation of mouse cells in rabbits. Our data indicate that the mouse eEPCs survived at least 7 days and did not cause an acute inflammatory response, whereas we observed a strong inflammatory response after transplantation of rat mature neonatal coronary endothelial cells in pigs and rabbits (our unpublished data). The survival time was sufficiently long to observe the beneficial effects on tissue recovery, arteriogenesis, and capillary density. Our previous data indicated that murine eEPCs are immunoprivileged in allogeneic settings due to lack of MHC I expression and resistance to nonactivated natural killer cells (7). The lack of acute inflammation in the xenotransplants could also be an advantage. A close inspection of the expression profile reveals that eEPCs express no measurable levels of genes encoding proinflammatory cytokines (such as IL-1, IL-2, IL-6, IL-23, etc.) that might account for this effect. A better understanding of the limited immunogenicity of embryonic cells could help design strategies using continuous and readily available sources for cell-based therapies.

ACKNOWLEDGMENTS

We would like to thank Dr. Christine Falk for useful discussions and Albert Geishauser, Susanne Helbig, and Elisabeth Ronft for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (DFG; Priority Program “Angiogenesis”) grants to A. K. Hatzopoulos, C. Kupatt, and P. Boekstegers, and the German Human Genome Project (DHGP) Grant No. 9907 and the National Genome Science Network (NGFN I) Grant No. 01GS0113 to A. K. Hatzopoulos.

REFERENCES

1. Kawamoto, A., Gwon, H.-C., Iwaguro, H., Yamaguchi, M., Uchida, S., Masuda, H., Silver, M., Ma, H., Kearney, M., Isner, J. M., et al. (2001) Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* **103,** 634–637

2. Masuda, H., and Asahara, T. (2003) Post-natal endothelial progenitor cells for neovascularization in tissue regeneration. *Cardiovasc. Res.* **58,** 390–398

3. Britten, M. B., Abolmaali, N. D., Assmus, B., Lehmann, R., Honold, J., Schmitt, J., Vogl, T. J., Martin, H., Schachinger, V., Dimmeler, S., et al. (2003) Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction

(TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation* **108,** 2212–2218

4. Wollert, K. C., Meyer, G. P., Lotz, J., Ringes-Lichtenberg, S., Lippolt, P., Breidenbach, C., Fichtner, S., Korte, T., Hornig, B., and Messinger, D. (2004) Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* **364,** 141–148

5. Hatzopoulos, A. K., Folkman, J., Vasile, E., Eiselen, G. K., and Rosenberg, R. D. (1998) Isolation and characterization of endothelial progenitor cells from mouse embryos. *Development* **125,** 1457–1468

6. Vajkoczy, P., Blum, S., Lamparter, M., Mailhammer, R., Erber, R., Engelhardt, B., Vestweber, D., and Hatzopoulos, A. K. (2003) Multistep nature of microvascular recruitment of ex vivo-expanded embryonic endothelial progenitor cells during tumor angiogenesis. *J. Exp. Med.* **197,** 1755–1765

7. Wei, J., Blum, S., Unger, M., Jarmy, G., Lamparter, M., Geishauser, A., Vlastos, G. A., Chan, G., Fischer, K. D., Rattat, D., et al. (2004) Embryonic endothelial progenitor cells armed with a suicide gene target hypoxic lung metastases after intravenous delivery. *Cancer Cell* **5,** 477–488

8. Koczulla, R., von Degenfeld, G., Kupatt, C., Krotz, F., Zahler, S., Gloe, T., Issbrucker, K., Unterberger, P., Zaiou, M., Lebherz, C., et al. (2003) An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J. Clin. Invest.* **111,** 1665–1672

9. Lebherz, C., von Degenfeld, G., Karl, A., Pfosser, A., Raake, P., Pachmayr, F., Scholz, D., Kupatt, C., and Boekstegers, P. (2003) Therapeutic angiogenesis/arteriogenesis in the chronic ischemic rabbit hindlimb: effect of venous basic fibroblast growth factor retroinfusion. *Endothelium* **10,** 257–265

10. Witzenbichler, B., Asahara, T., Murohara, T., Silver, M., Spyridopoulos, I., Magner, M., Principe, N., Kearney, M., Hu, J. S., and Isner, J. M. (1998) Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. *Am. J. Pathol.* **153,** 381–394

11. Kupatt, C., Wichels, R., Horstkotte, J., Krombach, F., Habazettl, H., and Boekstegers, P. (2002) Molecular mechanisms of platelet-mediated leukocyte recruitment during myocardial reperfusion. *J. Leukoc. Biol.* **72,** 455–461

12. Takahashi, T., Kalka, C., Masuda, H., Chen, D., Silver, M., Kearney, M., Magner, M., Isner, J. M., and Asahara, T. (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat. Med.* **5,** 434–438

13. Heil, M., Ziegelhoeffer, T., Mees, B., and Schaper, W. (2004) A different outlook on the role of bone marrow stem cells in vascular growth: bone marrow delivers software not hardware. *Circ. Res.* **94,** 573–574

14. Beckner, M. E., Jagannathan, S., and Peterson, V. A. (2002) Extracellular angio-associated migratory cell protein plays a positive role in angiogenesis and is regulated by astrocytes in coculture. *Microvasc. Res.* **63,** 259–269

15. Kryczek, I., Lange, A., Mottram, P., Alvarez, X., Cheng, P., Hogan, M., Moons, L., Wei, S., Zou, L., Machelon, V., et al. (2005) CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. *Cancer Res.* **65,** 465–472

16. Byrd, N., Becker, S., Maye, P., Narasimhaiah, R., St-Jacques, B., Zhang, X., McMahon, J., McMahon, A., and Grabel, L. (2002) Hedgehog is required for murine yolk sac angiogenesis. *Development* **129,** 361–372

17. Amin, M. A., Volpert, O. V., Woods, J. M., Kumar, P., Harlow, L. A., and Koch, A. E. (2003) Migration inhibitory factor mediates angiogenesis via mitogen-activated protein kinase and phosphatidylinositol kinase. *Circ. Res.* **93,** 321–329

18. Bock-Marquette, I., Saxena, A., White, M. D., DiMaio, J., and Srivastava, D. (2004) Thymosin [beta]4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. *Nature* **432,** 466-472

19. Eisenberg, L. M., and Eisenberg, C. A. (2004) An in vitro analysis of myocardial potential indicates that phenotypic plasticity is an innate property of early embryonic tissue. *Stem Cells Dev.* **13,** 614–624

20. Barandon, L., Couffinhal, T., Ezan, J., Dufourcq, P., Costet, P., Alzieu, P., Leroux, L., Moreau, C., Dare, D., and Duplaa, C. (2003) Reduction of infarct size and prevention of cardiac rupture in transgenic mice overexpressing FrzA. *Circulation* **108,** 2282–2289

21. Shi, Q., Rafii, S., Wu, M. H., Wijelath, E. S., Yu, C., Ishida, A., Fujita, Y., Kothari, S., Mohle, R., Sauvage, L. R., et al. (1998) Evidence for circulating bone marrow-derived endothelial cells. *Blood* **92,** 362–367

22. Corbel, S. Y., Lee, A., Yi, L., Duenas, J., Brazelton, T. R., Blau, H. M., and Rossi, F. M. (2003) Contribution of hematopoietic stem cells to skeletal muscle. *Nat. Med.* **9,** 1528–1532

23. Aicher, A., Brenner, W., Zuhayra, M., Badorff, C., Massoudi, S., Assmus, B., Eckey, T., Henze, E., Zeiher, A. M., and Dimmeler, S. (2003) Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation* **107,** 2134–2139

24. Jackson, K. A., Majka, S. M., Wang, H., Pocius, J., Hartley, C. J., Majesky, M. W., Entman, M. L., Michael, L. H., Hirschi, K. K., and Goodell, M. A. (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J. Clin. Invest.* **107,** 1395–1402

25. Kamihata, H., Matsubara, H., Nishiue, T., Fujiyama, S., Tsutsumi, Y., Ozono, R., Masaki, H., Mori, Y., Iba, O., Tateishi, E., et al. (2001) Implantation of bone marrow mononuclear

cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* **104,** 1046–1052

26. Shintani, S., Murohara, T., Ikeda, H., Ueno, T., Sasaki, K., Duan, J., and Imaizumi, T. (2001) Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* **103,** 897–903

27. Strauer, B. E., Brehm, M., Zeus, T., Kostering, M., Hernandez, A., Sorg, R. V., Kogler, G., and Wernet, P. (2002) Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* **106,** 1913–1918

28. von Degenfeld, G., Raake, P., Kupatt, C., Lebherz, C., Hinkel, R., Gildehaus, F. J., Munzing, W., Kranz, A., Waltenberger, J., Simoes, M., et al. (2003) Selective pressure-regulated retroinfusion of fibroblast growth factor-2 into the coronary vein enhances regional myocardial blood flow and function in pigs with chronic myocardial ischemia. *J. Am. Coll. Cardiol.* **42,** 1120–1128

29. Kupatt, C., Wichels, R., Deiss, M., Molnar, A., Lebherz, C., Raake, P., von Degenfeld, G., Hahnel, D., and Boekstegers, P. (2002) Retroinfusion of NFkappaB decoy oligonucleotide extends cardioprotection achieved by CD18 inhibition in a preclinical study of myocardial ischemia and retroinfusion in pigs. *Gene Ther*. **9,** 518–526

30. Kupatt, C., Hinkel, R., Vachenauer, R., Horstkotte, J., Raake, P., Sandner, T., Kreuzpointner, R., Muller, F., Dimmeler, S., Feron, O., et al. (2003) VEGF165 transfection decreases postischemic NF-kappa B-dependent myocardial reperfusion injury in vivo: role of eNOS phosphorylation. *FASEB J.* **17,** 705–707

31. Beck, H., Voswinckel, R., Wagner, S., Ziegelhoeffer, T., Heil, M., Helisch, A., Schaper, W., Acker, T., Hatzopoulos, A. K., and Plate, K. H. (2003) Participation of bone marrow-derived cells in long-term repair processes after experimental stroke. *J. Cereb. Blood Flow Metab.* **23,** 709–717

32. Ziegelhoeffer, T., Fernandez, B., Kostin, S., Heil, M., Voswinckel, R., Helisch, A., and Schaper, W. (2004) Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ. Res.* **94,** 230–238

33. Sato, N., Sanjuan, I. M., Heke, M., Uchida, M., Naef, F., and Brivanlou, A. H. (2003) Molecular signature of human embryonic stem cells and its comparison with the mouse. *Dev. Biol.* **260,** 404–413

34. Kinnaird, T., Stabile, E., Burnett, M. S., Lee, C. W., Barr, S., Fuchs, S., and Epstein, S. E. (2004) Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ. Res.* **94,** 678–685

Received November 17, 2004; accepted May 17, 2005.

Table 1

Factors secreted by the embryonic endothelial progenitor cells

Gene Name	Abbr.*	Exp. Val.†	Acc. No.‡
Angiogenesis-related factors			
angio-associated migratory protein	Aamp	3253.2	NM_146110
PDGFA associated protein 1	Pdap1	2702.5	—
vascular endothelial growth factor B	Vegfb	1996.9	NM_011697
platelet derived growth factor α	Pdgfa	826.4	NM_008808
vascular endothelial growth factor A	Vegfa	703.1	NM_009505
angiogenin	Ang	673.8	NM_007447
transforming growth factor α	Tgfa	522.3	NM_031199
angiopoietin-like 2	Angptl2	396.1	NM_011923
Chemokines			
chemokine-like factor super family 7	Cklfsf7	1556.3	NM_133978
chemokine-like factor super family 8	Cklfsf8	1296.1	NM_027294
chemokine-like factor super family 3	Cklfsf3	978.2	NM_024217
chemokine-like factor super family 6	Cklfsf6	781.5	NM_026036
chemokine (C-X-C motif) ligand 16	Cxcl16	487.8	NM_023158
chemokine (C-X-C motif) ligand 1	Cxcl1	431.9	NM_008176
chemokine-like factor super family 5	Cklfsf5	286.2	NM_026066
chemokine (C-X3-C motif) ligand 1	Cx3cl1	260.8	NM_009142
chemokine (C-C motif) ligand 24	Ccl24	251.1	NM_019577
chemokine (C-X-C motif) ligand 13	Cxcl13	249.6	NM_018866
Cytokines			
macrophage migration inhibitory factor	Mif	8745.1	NM_010798
interleukin 25	Il25	1644.5	NM_080837
interleukin 14	Il14	648.1	—
kit ligand	Kitl	489.2	NM_013598
tumor necrosis factor (ligand) superfamily, member 13	Tnfsf13	407.9	NM_023517
leukemia inhibitory factor	Lif	407.1	NM_008501
colony stimulating factor 1 (macrophage)	Csf1	403.7	NM_007778
interferon α family, gene 12	Ifna12	340.4	NM_177361
tumor necrosis factor (ligand) superfamily, member 12	Tnfsf12	272.7	NM_011614
interleukin 18 binding protein	Il18bp	244.8	NM_010531
Insulin family			
insulin-like growth factor binding protein 4	Igfbp4	3116.9	NM_010517
insulin-like growth factor 2, binding protein 1	Igf2bp1	2367.7	NM_009951
insulin-like growth factor 2, binding protein 3	Igf2bp3	1518.2	NM_023670
insulin-like growth factor binding protein 5	Igfbp5	649.5	NM_010518
insulin-like growth factor 1	Igf1	330.4	NM_010512 and NM_184052
BMPs/TGFbeta pathway			
follistatin	Fst	4494.9	NM_008046
bone morphogenetic protein 6	Bmp6	969.3	NM_007556
latent transforming growth factor β binding protein 3	Ltbp3	592.2	NM_008520
latent transforming growth factor β binding protein 4	Ltbp4	438.1	NM_175641
bone morphogenetic protein 2	Bmp2	379.7	NM_007553
BMP and activin membrane-bound inhibitor	Bambi	370.7	NM_026505
growth differentiation factor 9	Gdf9	329.4	NM_008110
follistatin-like	Fstl	318.1	NM_008047
inhibin β-A	Inhba	242.2	NM_008380
follistatin-like 3	Fstl3	237.5	NM_031380

wnt pathway			
connective tissue growth factor	Ctgf	6477.3	NM_010217
wingless-related MMTV integration site 11	Wnt11	1775.5	NM_009519
wingless-related MMTV integration site 7B	Wnt7b	1593.9	NM_009528
frizzled-related protein	Frzb	1428.3	NM_011356
wingless-related MMTV integration site 7A	Wnt7a	537.7	NM_009527
dickkopf homologue 3 (*Xenopus laevis*)	Dkk3	330.4	NM_015814
secreted frizzled-related sequence protein 4	Sfrp4	295.6	NM_016687
wingless-related MMTV integration site 4	Wnt4	252.4	NM_009523
secreted frizzled-related sequence protein 1	Sfrp1	263.1	NM_013834
secreted frizzled-related sequence protein 5	Sfrp5	248.8	NM_018780
wingless-type MMTV integration site 9A	Wnt9a	236.9	NM_139298
Miscellaneous growth factors			
thymosin, β 10	Tmsb10	12165.7	NM_025284
prothymosin α	Ptma	10004.1	NM_008972
thymosin, β 4, X chromosome	Tmsb4x	7172.9	NM_021278
hepatoma-derived growth factor	Hdgf	5922.8	NM_008231
Indian hedgehog	Ihh	905.6	NM_010544
chorionic gonadotropin β	Cgb	503.8	NM_053189
endothelial cell growth factor 1 (platelet-derived)	Ecgf1	429.6	NM_138302
hepatoma-derived growth factor, related protein 2	Hdgfrp2	349.7	NM_008233
neurturin	Nrtn	348.7	NM_008738
glia maturation factor, gamma	Gmfg	304.5	NM_022024
neuropeptide Y	Npy	262.4	NM_023456
fibroblast growth factor 3	Fgf3	258.4	NM_008007
glycoprotein hormone α 2	Gpha2	254.9	NM_130453
hepatoma-derived growth factor, related protein 3	Hdgfrp3	241.2	NM_013886
sonic hedgehog	Shh	237.7	NM_009170
Protease activity regulators			
tissue inhibitor of metalloproteinase 2	Timp2	6447.9	NM_011594
serine protease inhibitor, Kazal type 3	Spink3	4502.2	NM_009258
plasminogen activator, tissue	Plat	3494.9	NM_008872
a disintegrin and metalloprotease domain 10	Adam10	3042.1	NM_007399
serine protease inhibitor, Kunitz type 2	Spint2	2930.7	NM_011464
tissue factor pathway inhibitor	Tfpi	2368.1	NM_011576
tissue inhibitor of metalloproteinase 1	Timp1	2071.5	NM_011593
tissue inhibitor of metalloproteinase 3	Timp3	1074.2	NM_011595
bone morphogenetic protein 1	Bmp1	759.9	NM_009755
serine protease inhibitor, Kunitz type 1	Spint1	715.9	NM_016907
a disintegrin-like and metalloprotease motif 15	Adamts15	597.9	—
matrix metalloproteinase 14 (membrane-inserted)	Mmp14	416.1	NM_008608
a disintegrin-like and metalloprotease motif 9	Adamts9	385.6	—
matrix metalloproteinase 24	Mmp24	333.5	NM_010808
matrix metalloproteinase 15	Mmp15	234.5	NM_008609
matrix metalloproteinase 2	Mmp2	172.5	NM_008610

*Gene name abbreviation; †experimental value is the average of 3 independent experiments; ‡gene accession number. Only genes with values >230 are shown (except Mmp2). When a gene was represented more than once in the chip, the highest value is depicted. Genes in each group are sorted by expression level (highest on top).

Fig. 1

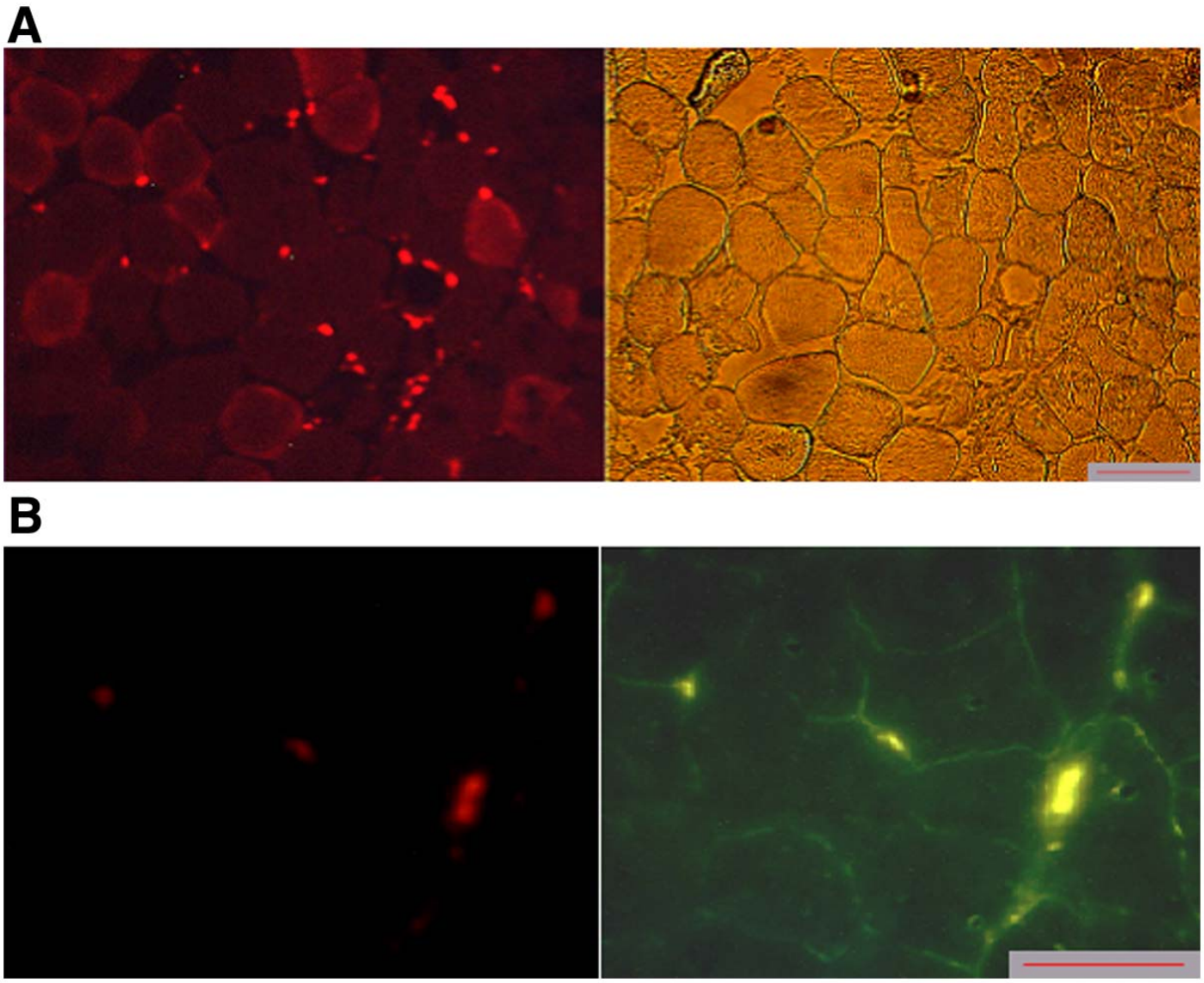

Fig. 1 (cont)

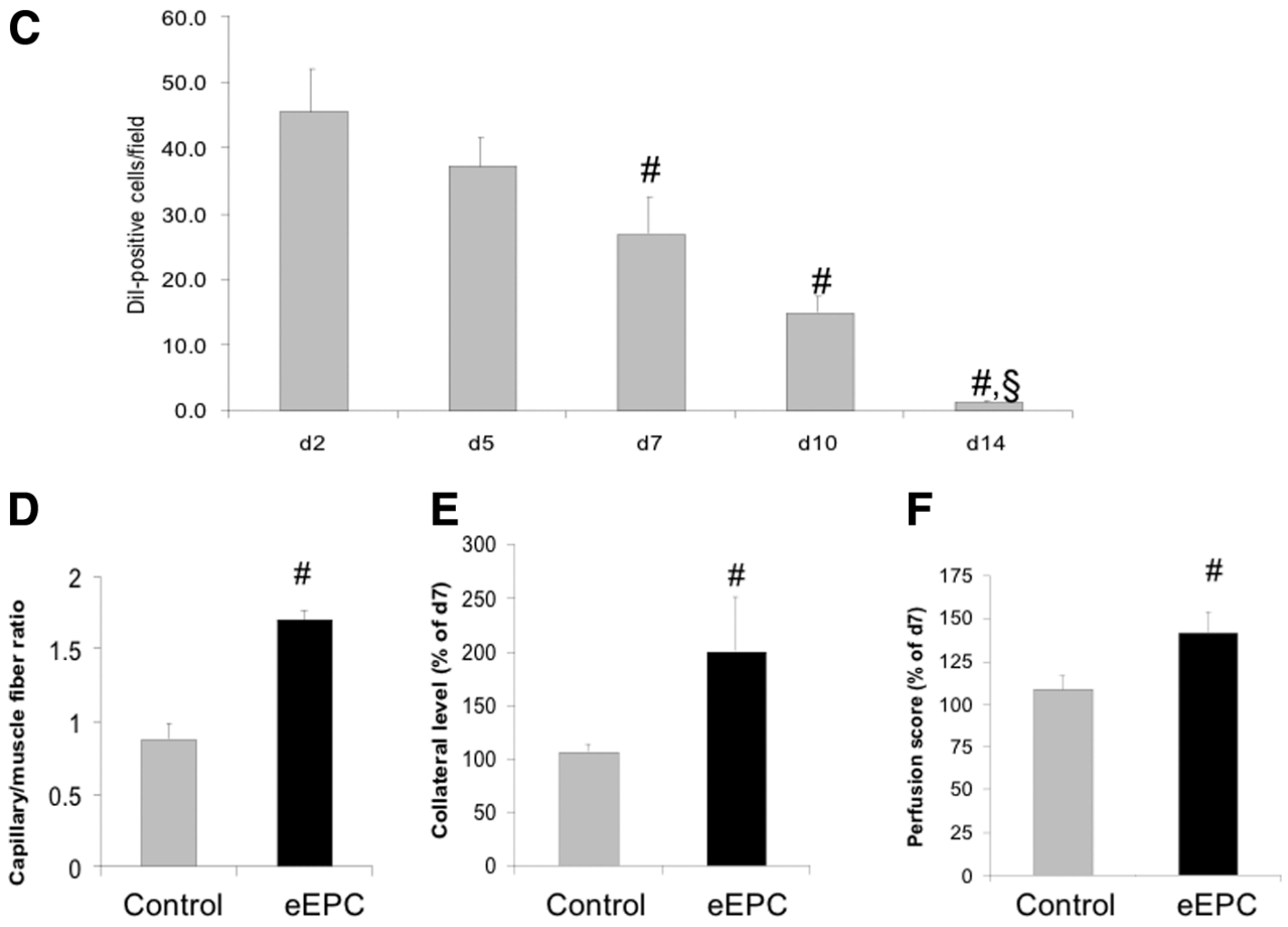

Figure 1. eEPCs application augments vascularization and improves circulation in chronic ischemic rabbit hindlimbs. ***A*)** 5×10^6 DiI-labeled eEPCs were applied via retroinfusion into hindlimbs of rabbits a week after excision of the femoral artery. The M. gastrognemius (M. gc.) was excised 5 days later, sectioned, and examined for the presence of engrafted cells using fluorescence microscopy. Transplanted eEPCs could be detected within and around vascular structures of muscle tissue (***left panel***). ***Right panel***: phase contrast picture of same area (bar=100 µm). ***B*)** DiI-labeled eEPCs (***left panel***, red) associate with host vasculature (green in merged picture, ***right panel***). Host endothelium was stained by injection of FITC-conjugated Helix pomatia lectin 5 min before tissue isolation (bar=100 µm). ***C*)** Quantification of eEPCs present within the M.gc. that was excised 2, 5, 7, 10, and 14 days (d) after eEPC retroinfusion (n=3 per time point, #P<0.05 vs. d2; $^{\S}P$<0.05 vs. all other time points). ***D*)** Quantification of capillaries in 3 muscle areas (M. gastrognemius, M. tibialis anterior, M. adductor) reveals an increase in capillary to muscle fiber ratios 28 days after eEPC treatment (d35). ***E*)** Collateral vessel formation increased in eEPC-treated rabbit hindlimbs as compared with untreated controls (changes, measured at d35, are given in % of d7 values). ***F*)** Angiography of rabbit hindlimbs after injection of a contrast agent at d35 shows a marked improvement in the perfusion score of eEPC-treated animals as compared with untreated controls. ***D–F*)** #P < 0.05 vs. control; n = 5 animals per group.

Fig. 2

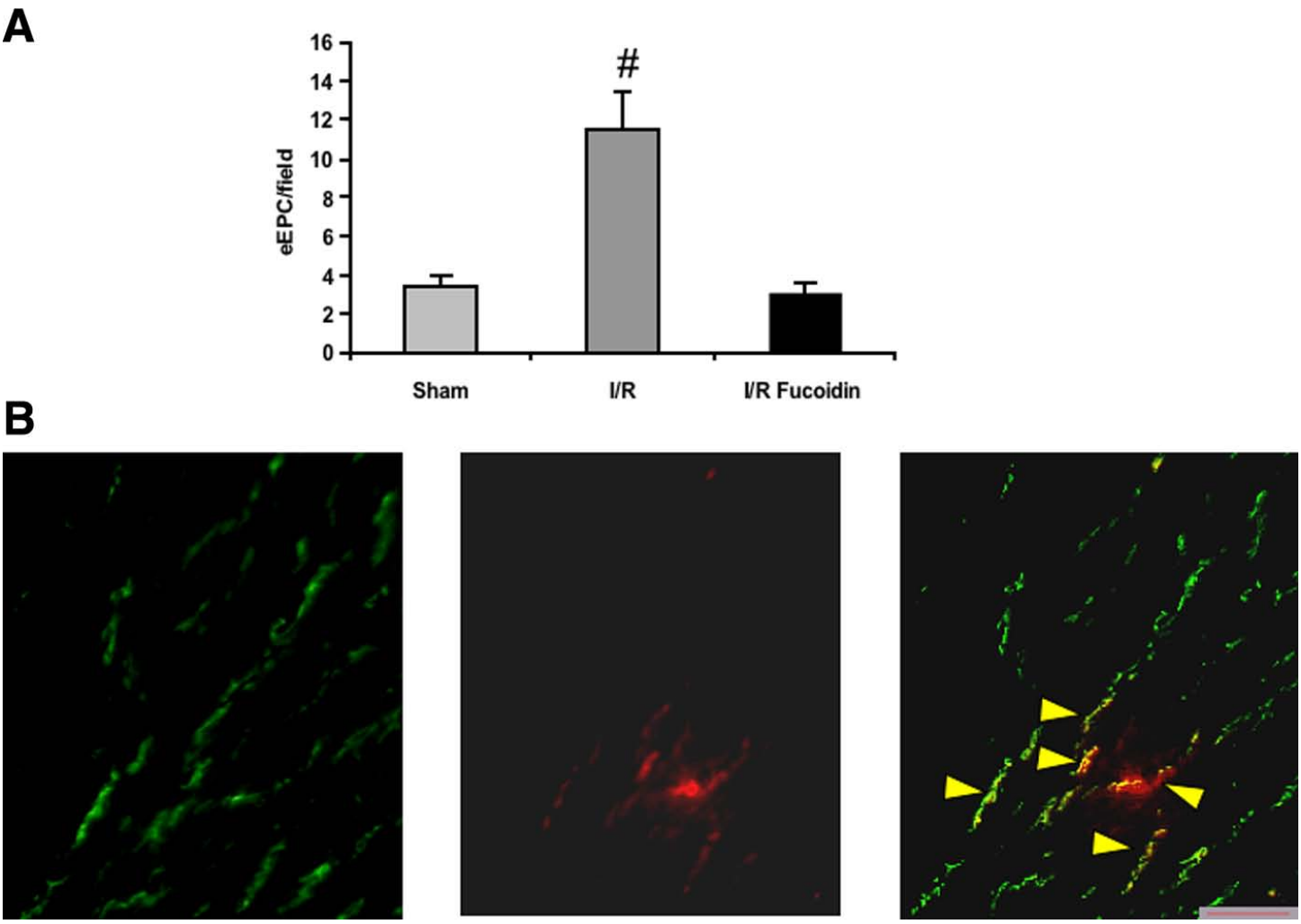

Fig. 2 (cont)

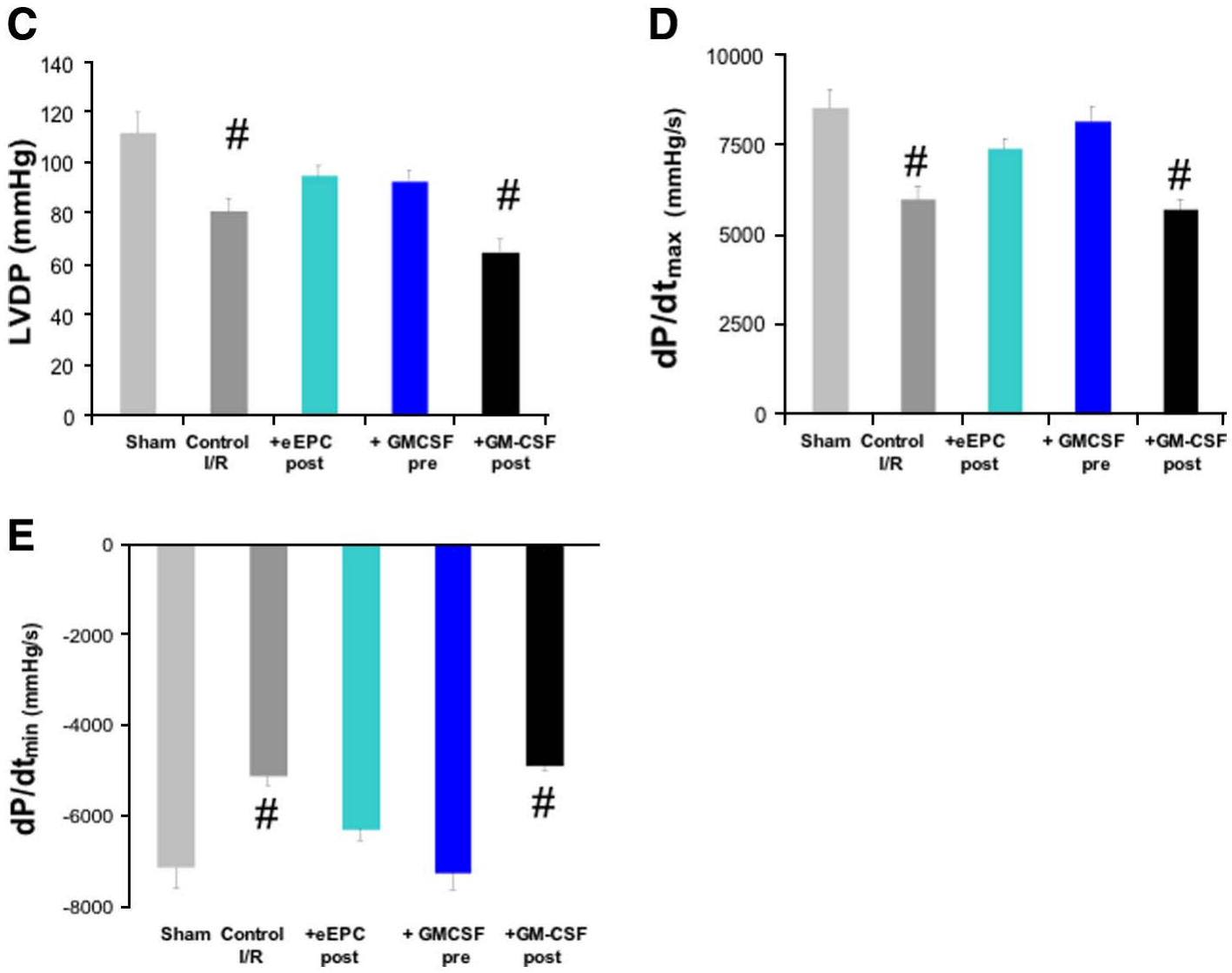

Figure 2. Systemic administration of eEPCs via the tail vein improves heart output in mice after myocardial infarction. ***A***) eEPCs are specifically retained in heart ischemic tissue. After ligation of left anterior descending coronary artery for 20 min, DiI-labeled eEPCs were injected into the external jugular vein. After 15 min reperfusion, hearts were isolated and washed and the number of eEPCs retained in ischemic areas was directly counted under a fluorescence microscope (I/R). Sham operated mice, without artery ligation, served as a control. Pretreatment with fucoidin blocked eEPC retention (I/R fucoidin; n=5 per group). ***B***) eEPCs associate with vascular structures in ischemic heart. DiI-labeled eEPCs were injected into tail vein 24 h after ischemia/reperfusion. Fuorescently labeled eEPCs were detected at d7 after ischemia (***mid-panel***, red color). Endothelial staining was performed by FITC-conjugated Helix pomatia lectin, injected iv 5 min before heart tissue isolation (***left panel***, green color). Merged images indicate integration of eEPCs (orange color) in vasculature (***right panel***, yellow arrowheads; bar=50 μm). ***C***) Systemic infusion of 3×10^5 eEPCs (+ EPC post) attenuates I/R injury as compared with saline infusion (control I/R) by improving LVDP as measured 14 days after transplantation. The effect was similar to GM-CSF treatment (0.5 μg/d ip injection for 10 days, starting at 3 days before infarction (+GM-CSF pre) but superior to GM-CSF treatment applied only in the 7 days post-I/R (GM-CSF post). Sham-operated mice served as controls. dP/dt_{max} (***D***) and dP/dt_{min} (***E***) evaluation 14 days after eEPC administration. Both parameters indicate improvement in heart output after eEPC-treatment or GM-CSF-pretreatment but not after postischemic GM-CSF application (#P<0.05 vs. sham; n=8 per group).

Fig. 3

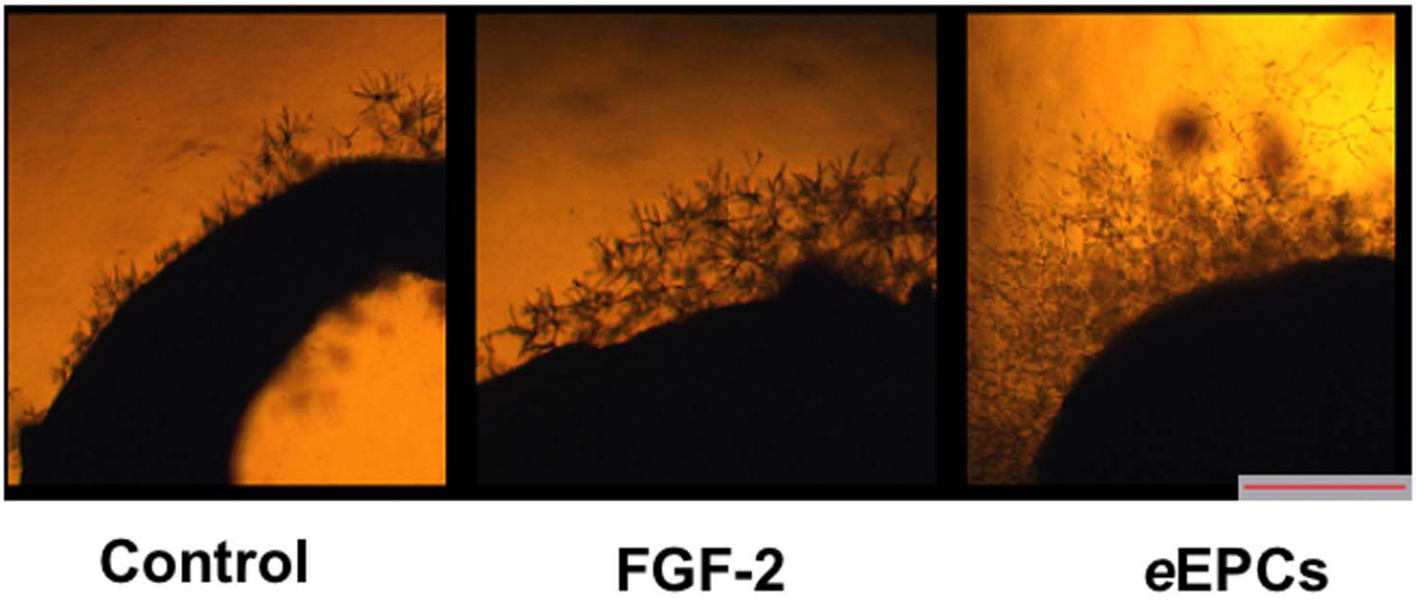

Figure 3. eEPCs induce angiogenesis ex vivo. Rabbit aortic rings were placed in Matrigel. After polymerization, control medium or medium containing 10 µg/ml FGF-2 or 5×10^4 eEPCs was placed on top. eEPCs or FGF-2 induced extensive capillary networks as compared with control aortic rings that received just medium (representative example of 3 independent experiments).

Fig. 4

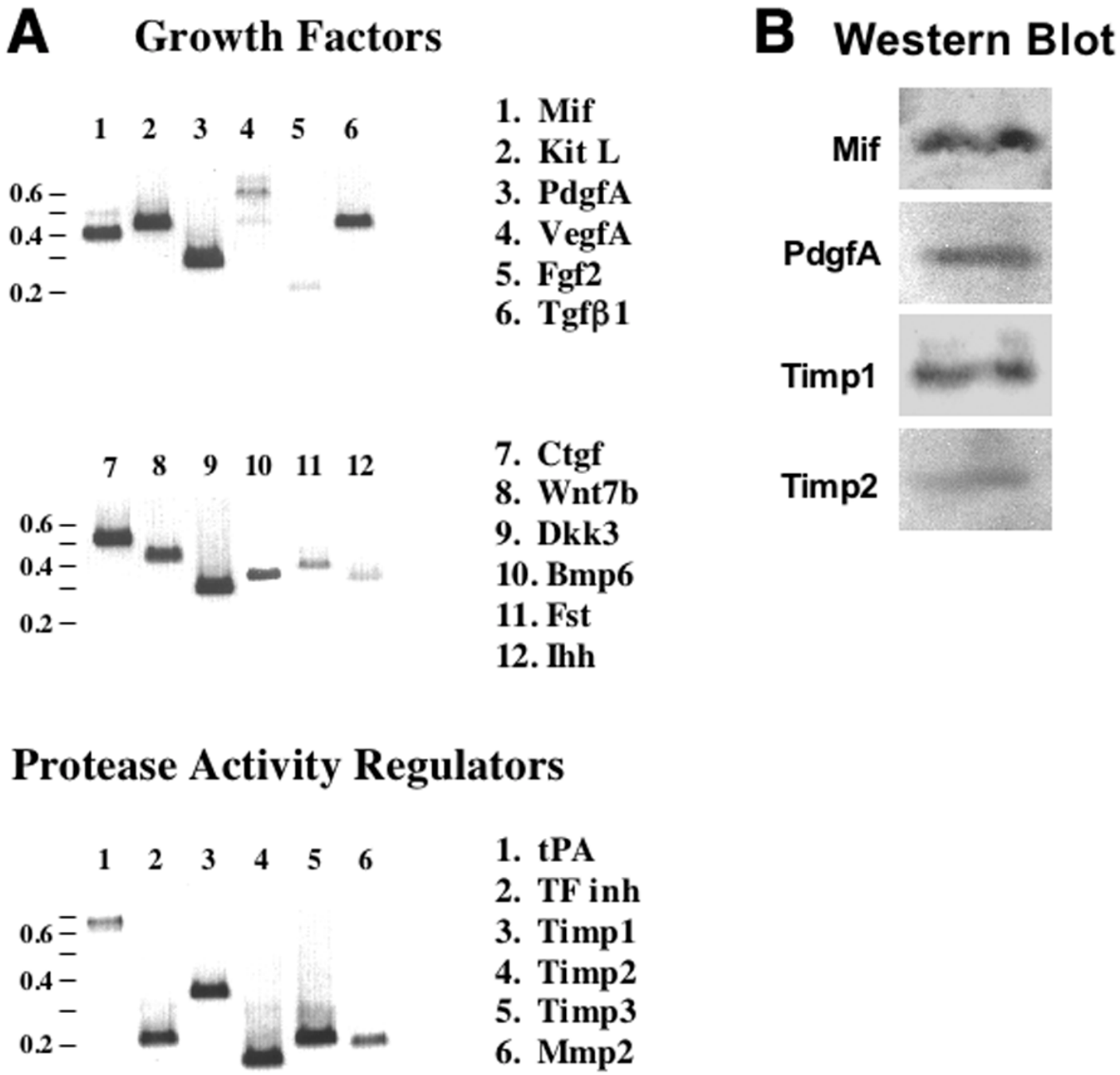

Figure 4. Embryonic EPCs express a wide spectrum of molecules that regulate angiogenesis and tissue recovery. ***A*)** Gene expression in eEPCs by RT-PCR using a panel of gene-specific primers. Molecular weight DNA ladder in kilobases is indicated on ***left***. Mif, macrophage migration inhibitory factor; Kit L, c-Kit ligand: stem cell factor; Pdgf A: platelet derived growth factor A; Vegf A: vascular endothelial growth factor A; Fgf2: fibroblast growth factor 2 (basic); Tgfβ1: transforming growth factor β1; Ctgf: connective tissue growth factor; Dkk3: dickkopf 3; Bmp6: bone morphogenetic protein 6; Fst: follistatin; Ihh: Indian hedgehog; tPA: tissue plasminogen activator; TF inh: tissue factor pathway inhibitor; Timp1, 2, 3: tissue inhibitor of metalloproteinases 1, 2, 3; Mmp2: matrix metalloproteinase 2. ***B*)** Protein production in eEPCs. Affymetrix data were validated for a subset of proteins by Western blot analysis using a panel of specific antibodies as indicated.