Estrogen Receptors α and β Mediate Contribution of Bone Marrow–Derived Endothelial Progenitor Cells to Functional Recovery After Myocardial Infarction

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Background—Estradiol (E_2) modulates the kinetics of circulating endothelial progenitor cells (EPCs) and favorably affects neovascularization after ischemic injury. However, the roles of estrogen receptors α (ERα) and β (ERβ) in EPC biology are largely unknown.

Methods and Results—In response to E_2, migration, tube formation, adhesion, and estrogen-responsive element–dependent gene transcription activities were severely impaired in EPCs obtained from ERα-knockout mice (ERαKO) and moderately impaired in ERβKO EPCs. The number of ERαKO EPCs (42.4±1.5; P<0.001) and ERβKO EPCs (55.4±1.8; P=0.03) incorporated into the ischemic border zone was reduced as compared with wild-type (WT) EPCs (72.5±1.3). In bone marrow transplantation (BMT) models, the number of mobilized endogenous EPCs in E_2-treated mice was significantly reduced in ERαKO BMT (WT mice transplanted with ERαKO bone marrow) (2.03±0.18%; P=0.004 versus WT BMT) and ERβKO BMT (2.62±0.07%; P=0.02 versus WT) compared with WT BMT (2.87±0.13%) (WT to WT BMT as control) mice. Capillary density at the border zone of ischemic myocardium also was significantly reduced in ERαKO BMT and ERβKO BMT compared with WT mice (WT BMT, 1718±75/mm²; ERαKO BMT, 1107±48/mm²; ERβKO BMT, 1567±50/mm²). ERα mRNA was expressed more abundantly on EPCs compared with ERβ. Moreover, vascular endothelial growth factor was significantly downregulated on ERαKO EPCs compared with WT EPCs both in vitro and in vivo.

Conclusions—Both ERα and ERβ contribute to E_2-mediated EPC activation and tissue incorporation and to preservation of cardiac function after myocardial infarction. ERα plays a more prominent role in this process. Moreover, ERα contributes to upregulation of vascular endothelial growth factor, revealing possible mechanisms of an effect of E_2 on EPC biology. Finally, these data provide additional evidence of the importance of bone marrow–derived EPC phenotype in ischemic tissue repair. (Circulation. 2006;114:2261-2270.)

Key Words: angiogenesis • bone marrow cells • hormones • myocardial infarction • receptors, estrogen
was associated with reduced risk of mortality after myocardial infarction (MI).12 Tissue ischemia induces upregulation of angiogenic growth factors and mobilization of circulating cellular elements that together enable development of an accessory vasculature for organ survival. Recently, endothelial progenitor cells (EPCs) isolated from peripheral blood have been shown to incorporate into foci of neovascularization in the adult, ie, postnatal vasculogenesis,13,14 These circulating EPCs are derived from bone marrow and mobilized endogenously in response to tissue ischemia or exogenously by cytokine stimulation.15–18 Previous findings have suggested that estrogen also could augment the recruitment of EPCs for vascular repair.11,19,20 Our laboratory showed that the effects of estrogen on EPC recruitment in vascular repair were endothelial nitric oxide synthase dependent.11,20 However, the potential role of estrogen receptor in EPC recruitment for myocardial microvascular repair has not been studied. In this study, we investigated the roles of ERα and ERβ in estrogen-induced, EPC-mediated tissue repair in the setting of acute MI.

Methods

Cell Culture
Mononuclear cells were isolated from mouse bone marrow and plated for EPCs as described previously21,22 and in the online Data Supplement. The rationale for using a culture method to enrich the EPC fraction of bone marrow cells is as follows: For phenotyping and assessing gene expression, circulating cells in the mouse are too scarce, thus mandating the use of a more abundant source of cells. A method has been developed and used repeatedly in our laboratory (and in multiple peer-reviewed publications35,16,23) that favors the growth of cells with endothelial lineage capability while diminishing the non-EPC population. Medium was replaced with phenol red–free medium (EBM, Cambrex, Walkersville, Md) with 5% charcoal-dextran–treated fetal bovine serum (Biosource, Invitrogen, Carlsbad, Calif) at day 5 to remove the effects of estrogen-like activity of phenol red and estrogen derived from the serum. After 2 days in further culture, the cells were used as an EPC-rich cell population for cell function studies and real-time polymerase chain reaction (RT-PCR).

In Vitro Cell Function Assays (Proliferation, Migration, Tube Formation, and Adhesion Activity)
Cell proliferation was assessed by [3H]thymidine incorporation into DNA as described before.24 Migration was measured in a modified Boyden’s chamber. Tube formation assay was performed as described before using Matrigel-Matrix (BD Biosciences, San Jose, Calif) and assessing gene expression, circulating cells in the mouse are too scarce, thus mandating the use of a more abundant source of cells. A method has been developed and used repeatedly in our laboratory (and in multiple peer-reviewed publications35,16,23) that favors the growth of cells with endothelial lineage capability while diminishing the non-EPC population. Medium was replaced with phenol red–free medium (EBM, Cambrex, Walkersville, Md) with 5% charcoal-dextran–treated fetal bovine serum (Biosource, Invitrogen, Carlsbad, Calif) at day 5 to remove the effects of estrogen-like activity of phenol red and estrogen derived from the serum. After 2 days in further culture, the cells were used as an EPC-rich cell population for cell function studies and real-time polymerase chain reaction (RT-PCR).

Animals
All mice used in this study were handled in accordance with the guidelines of the Animal Care and Use Committee at St Elizabeth’s Medical Center of Boston (Mass). Female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) were used as WT mice. Full details about ERα-null mutant mice and ERβ-null mutant mice have been given recently.26 In ERα-null mutant mice, exon 3 of ERα was deleted, and no ERα proteins were found in this strain. In ERβ-null mutant mice, exon 3 of ERβ was deleted, and no ERβ proteins were found in this strain. These mice are of C57BL/6 background.

Bone Marrow Transplantation Model
Female C57BL/6J mice 9 to 10 weeks old were studied. Mice underwent ovariectomy at day −28, WT, ERαKO, or ERβKO mice 8 to 12 weeks old were used as donors of the bone marrow. The BMT procedure was performed as described previously.16,21 At day −7, by which time the bone marrow of the recipient mice was reconstituted, BMT mice received either 17b-estradiol (E2) pellets (Innovative Research of America, Sarasota, Fla) or placebo-containing pellets implanted subcutaneously into the dorsal neck region of the animals. To achieve typical E2 levels found at mid-cycle, a 90-day release pellet containing 1.7 mg E2 was used. Circulating E2 levels in mice with the E2 pellets and in mice with the placebo pellets were previously reported.28 Seven days later (day 0), animals underwent MI surgery.

Surgical Procedure
MI was induced by permanent left anterior descending coronary artery ligation as described previously29 using intraperitoneal injection of avertin 0.015 mg/kg and assisted ventilation (Harvard Apparatus, Holliston, Mass).

In Vivo Tissue Homing Assay
Female C57BL/6J mice 9 to 10 weeks old underwent ovariectomy at day −28, followed by either 1.7 mg E2 pellet or placebo-containing pellet implantation, together with splenectomy at day −7. Seven days later (day 0), animals underwent MI surgery. Cultured EPCs were coincubated with 2 μg/mL Dil-ac-LDL (Biomedical Technologies, Stoughton, Mass) for 1 hour, and 5×105 EPCs were injected intravenously immediately after MI surgery. Hearts of these mice were harvested at the indicated time after MI surgery for histology.

Fluorescence-Activated Cell Sorting Analysis
To evaluate the number of circulating EPCs, 1 mL blood was taken at days −1, 7, and 28, and mononuclear cells were isolated by density centrifugation with Histopaque-1083 (Sigma-Aldrich, St Louis, Mo) for fluorescence-activated cell sorting analysis (Becton Dickinson, Franklin Lakes, NJ). The viable mononuclear cell population (2 to 4×105 cells were available from 1 mL blood) was analyzed for the expression of Sca-1–FITC (BD PharMingen, San Diego, Calif) and Flk-1–PE (BD PharMingen). Isotype-identical antibodies served as negative controls (Jackson ImmunoResearch, West Grove, Pa).

Echocardiography
Left ventricular function was assessed by transthoracic echocardiography (SONOS 5500, Hewlett Packard, Palo Alto, Calif) at days −1, 7, 14, 21, and 28. Left ventricular end-diastolic dimension, left ventricular end-systolic dimension, and fractional shortening at the papillary muscle level of the left ventricle were measured, and the mean value of 3 measurements was determined for each sample.

Histological Analysis
In BMT models, hearts were harvested at day 28 for histological analysis. The explanted hearts were sliced in a bread-loaf fashion into transverse sections from apex to base and fixed with 4% paraformaldehyde. Tissues were stained for Masson’s trichrome staining to measure the average ratio of fibrosis area to total left ventricular area.

Immunohistochemistry
The hearts of treated mice were harvested at predetermined times after surgery and frozen in optical coherence tomography compound (Sakura Finetek USA, Inc, Torrance, Calif). For capillary detection, sections were stained with mouse anti-CD31 antibody (BD Pharmingen). For detection of vascular endothelial growth factor (VEGF), sections were incubated with mouse anti-VEGF antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif). Details are described in the Data Supplement.

Quantitative RT-PCR
RNA was collected from 8×105 cells per sample with RNA STAT-60 (TEL-TEST, Inc, Friendswood, Texas). Total RNA was reverse transcribed with iScript cDNA Synthesis Kit (Bio-Rad).
Determination of E₂-Surface Binding With E₂-Conjugated BSA-FITC
E₂-ER binding study was performed as previously described²⁷ with modification, as described in the Data Supplement.

Reporter Gene Luciferase Assay for Estrogen-Responsive Element-Dependent Transcription
EPCs were transiently transfected with estrogen-responsive element (ERE)-luciferase reporter construct using Fusene 6 transfection reagent (Roche, Palo Alto, Calif) according to the manufacturer’s instructions, and luciferase activity was determined as described before.²⁸

Statistical Analysis
All values are expressed as mean±SE. Statistical significance was evaluated through the use of an unpaired t test for comparisons between 2 groups. For comparison among 3 or 4 groups, 1-factor analysis of variance was used, followed by an unpaired t test to compare 2 groups within them. When multiple time-point measurements were taken, repeated-measures analysis was done, followed by an unpaired t test. A value of P<0.05 was considered statistically significant.

Results

E₂ Effects on EPC Cell Function Are Abolished in ER-Knockout EPCs
First, we evaluated the role of ER expression on EPC cell function in vitro. In migration assays (Figure 1a), EPC migration was significantly increased in WT cells by physiological concentrations of E₂ but was absent in ER KO EPCs and reduced in ER βKO EPCs (WT EPCs: 8.89±0.17% E₂ 0 mol/L, 14.1±1.50% E₂ 10⁻⁹ mol/L, P<0.01 versus without E₂; 14.8±0.81% E₂ 10⁻⁸ mol/L, P<0.01 versus without E₂; ER βKO EPCs: 10.6±0.20% E₂ 0 mol/L, 13.4±1.74% E₂ 10⁻⁸ mol/L, P<0.01 versus without E₂). In proliferation assays (Figure 1b), E₂-induced proliferation of in vitro expanded EPCs obtained from both ER αKO and ER βKO mice was significantly reduced compared with EPCs obtained from WT littermates (WT EPCs: 8540±420 cpm/E₂ 10⁻⁸ mol/L versus ER αKO EPCs: 4320±50 cpm/E₂ 10⁻⁸ mol/L, P<0.001; WT EPCs: 8540±420 cpm/E₂ 10⁻⁸ mol/L versus ER βKO EPCs: 4650±240 cpm/E₂ 10⁻⁸ mol/L, P<0.001).

Similarly, adhesion activity to vitronectin, which is increased in a dose-dependent manner by E₂ in WT EPCs, was absent in ER αKO EPCs and reduced in ER βKO EPCs (Figure 1c) (WT EPCs: 1.00±0.03/E₂ 0 mol/L, 1.27±0.04/E₂ 10⁻⁹ mol/L, P<0.05 versus without E₂; 1.57±0.06/E₂ 10⁻⁸ mol/L, P<0.001 versus without E₂; ER αKO EPCs: E₂ 0 mol/L, 1.00±0.19; E₂ 10⁻⁸ mol/L: 0.89±0.03 (P=NS versus 0 mol/L), E₂ 10⁻⁹ mol/L: 1.02±0.08 (P=NS versus 0 mol/L); ER βKO EPCs: 1.00±0.06/E₂ 0 mol/L, 1.23±0.04/E₂ 10⁻⁹ mol/L, P<0.001 versus without E₂, 1.24±0.10/E₂ 10⁻⁸ mol/L, P<0.01 versus without E₂). Finally, we evaluated tube formation, an established method to assess functional angiogenic activity in vitro. Capillary-like tube formation requires several biological activities such as endothelial cell proliferation, cell migration, protease secretion, and cell-to-cell interaction. As shown in Figure 1d and 1e, WT EPCs showed an E₂ concentration-dependent response in capillary network formation (21.8±0.8 mm/E₂ 0 mol/L, 114±4.2 mm/E₂ 10⁻⁹ mol/L, P<0.001 versus without E₂, 219±8.4 mm/E₂ 10⁻⁸ mol/L, P<0.01 versus without E₂). Under similar culture condition, ER βKO EPCs (97.8±5.9 mm/E₂ 10⁻⁸ mol/L) made fewer networks compared with WT EPCs (P<0.001 versus WT EPCs), and tube formation by ER αKO EPCs (18.0±0.8 mm/E₂ 10⁻⁸ mol/L) was severely impaired (P<0.001 versus WT EPCs).

E₂ Contribution to EPC Tissue Homing Is Impaired in ER-Knockout EPCs
From the results of the in vitro cell function assays, we hypothesized that E₂ could modulate chemotactic activity in EPCs via both ERs. Accordingly, we evaluated EPC homing in vivo in a tissue homing assay using mouse acute MI models. Incorporation of Dil-labeled WT EPCs, injected just after MI induction, was observed in the border zone of the ischemic myocardium (Figure 2a) at day 3 (36.7±3.4 cells/×200 field). At day 5, the number of EPCs at the site increased (63.6±2.5 cells/×200 field) and persisted until at least day 10 (day 7, 65.7±4.0 cells; day 10, 61.5±4.0 cells; Figure 2b). Next, we compared WT, ER αKO, and ER βKO EPC incorporation at the border zone of the ischemic myocardium at day 7, choosing this time point as the approximate peak on the basis of pilot studies. Incorporation of WT EPCs in ovariectomized mice with placebo pellets was used as a negative control (40.2±3.7 cells). As shown in Figure 2c and 2d, the number of incorporated cells per ×200 magnification field was significantly higher in WT EPCs (72.5±1.3) compared with ER αKO (42.4±1.5; P<0.001) and ER βKO EPCs (55.4±1.8; P=0.03).

E₂ Contribution to EPC Mobilization From Bone Marrow Was Impaired From ER-Knockout Bone Marrow
To evaluate the effect of ER-mediated effects on circulating EPC kinetics in vivo, peripheral blood was collected at serial time points after MI in WT, ER αKO, and ER βKO BMT models and prepared for fluorescence-activated cell sorting analysis. The light-scatter pattern of mononuclear cells was similar in WT, ER αKO, and ER βKO cells (Figure 3a). As shown in Figure 3b, a significantly greater number of circulating Sca-1⁺/Flk-1⁺ cells were observed in WT BMT mice 1 week after MI (2.87±0.13% of total mononuclear cells) compared with ER αKO (2.03±0.18%; P=0.004 versus WT BMT with E₂ pellet) and ER βKO (2.62±0.07%; P=0.02 versus WT BMT with E₂ pellet) BMT mice.

Protective Effect of E₂ in MI Is Reduced in ER-Knockout BMT Model
Physiological and histological assessments were then performed after MI in WT, ER αKO, and ER βKO BMT mice. WT mice with WT BMT plus placebo pellets were evaluated as negative controls. Left ventricular end-diastolic dimensions and systolic function were not significantly different...
between WT BMT and ER mutant BMT mice early after MI (Figure 4a). However, beginning 3 weeks after MI, echocardiography revealed better preservation of fractional shortening in the WT BMT mice compared with ERαKO BMT mice (WT BMT versus ERαKO BMT: \( P < 0.02 \) at 3 weeks after MI, \( P = 0.007 \) at 4 weeks after MI; Figure 4a, left).

Masson’s trichrome-stained tissues in ERαKO and ERβKO BMT mice indicated marked dilation of the left ventricular cavity consistent with the echocardiographic measurements (Figure 4b). The area of fibrosis was significantly less in WT BMT mice than in ERαKO and ERβKO BMT mice (WT BMT, 13.5±1.1%; ERαKO BMT, 19.4±2.4%; ERβKO BMT, 17.9±1.1%; Figure 4c).

WT BMT mice with placebo pellets were analyzed as negative controls (WT BMT with placebo, 21.7±0.8%). Capillary density at the border zone of ischemic myocardium 4 weeks after MI was significantly greater in WT BMT mice with \( E_2 \) compared with ERαKO BMT and ERβKO BMT mice with \( E_2 \) (WT BMT, 1718±75/mm²; ERαKO BMT, 1107±48/mm²; ERβKO BMT, 1567±50/mm²; Figure 4d and 4e). In WT BMT mice with placebo pellets, capillary density was 1136±83/mm².

**ERα/ERβ Expression and Binding Activity to \( E_2 \) on Mouse EPCs**

In the above series of experiments, ERαKO EPCs appeared to have a more prominent phenotype than ERβKO EPCs. To better understand the potential mechanisms for...
this, we first evaluated ERα and ERβ mRNA expression in WT mouse EPCs by RT-PCR. Both receptors were expressed on EPCs from WT mice cultured for 7 days (Figure 5a). Next, we used quantitative RT-PCR to evaluate the relative expression of individual receptors in EPCs and showed that ERα mRNA was expressed more abundantly on WT EPCs compared with ERβ mRNA (relative expression versus 18S: ERα, 1.88±0.18, ERβ, 0.01±0.01; Figure 5b and 5c; note that the y-axis scale is 10X between Figure 5b and Figure 5c). Because ERβ expression was low by RT-PCR, we reevaluated it using other primer sets that were within exon 5 of the ERβ sequence (Figure 5c, right). In WT EPCs, the relative expression of ERβ versus 18S was again much lower than ERα expression (0.12±0.03). In ERβKO mice, ERβ exon 5 gene transcription was accelerated (13.2±0.27), which indicates that the gene was actually present in ERβKO mice except exon 3, where the ERβ gene was disrupted. In addition, E2 10^(-8) mol/L induces ERα mRNA upregulation in WT EPCs (P=0.004) and ERβKO EPCs (P=0.015; Figure 5d).

**Figure 2.** In vivo EPC tissue homing assay. a, Histology at the border zone of ischemic myocardium on indicated days after MI surgery when WT EPCs were injected into an E2 pellet–implanted WT mouse. Injected EPCs are labeled with Dil-acLDL (red). Nuclear is stained with DAPI (blue). Scale bar represents 100 μm. b, Number of Dil-positive cells at the border zone was counted and shown as mean±SE n=5. c, Histology at the border zone of ischemic myocardium at day 7 after MI surgery when WT, ERαKO, and ERβKO EPCs were injected into an E2 pellet–implanted WT mouse. Samples of WT EPC injection into WT mice with placebo pellets are used as negative controls. d, Quantification of injected, labeled EPCs in the MI border zone shown as mean±SE. n=5. WT E2+ indicates WT EPC injection into WT mouse with E2 pellet; WT E2−, WT EPC injection into WT mouse with placebo pellet; ERαKO E2+, ERαKO EPC injection into WT mouse with E2 pellet; ERβKO E2+, ERβKO EPC injection into WT mouse with E2 pellet. *P<0.001, +P=0.03 vs WT E2+.

**Figure 3.** EPC mobilization from bone marrow in response to MI in WT, ERαKO, and ERβKO BMT to WT mice. a, Forward scatter (FSC-H) and side scatter (SSC-H) plot for circulating blood mononuclear cells in steady-state WT, ERαKO, and ERβKO mice. Numbers show percent of data-collected cells per whole cells (circled area). b, Percentage of Sca-1+/Flk-1+ mononuclear cells in circulating blood before and 1 and 4 weeks after MI is indicated as mean±SE. WT E2+ indicates WT BMT to WT mouse with E2 pellet (n=12); WT E2−, WT BMT to WT mouse with placebo pellet (n=12); ERαKO E2+, ERαKO BMT to WT mouse with E2 pellet (n=8); ERβKO E2+, ERβKO BMT to WT mouse with E2 pellet (n=8). *P=0.004, +P=0.02.
Bovine serum albumin–FITC–tagged estradiol (E2coBSA-FITC) was used to investigate estrogen binding in WT, ERαKO, and ERβKO EPCs (Figure 5e and 5f). After 4 hours of incubation, 64.5±4.1% of cells were positive for FITC in WT EPCs, whereas 11.4±2.9% of cells were positive in ERαKO EPCs (P<0.001).

There were no significant differences in binding activity between WT and ERβKO EPCs (58.8±3.0%). Preincubation with unlabeled E2 10^−6 mol/L diminished E2coBSA binding (20.8±2.5%), indicating ligand specificity. As an additional measure of individual ER function, ERE-dependent transcription of the reporter gene luciferase was evaluated in EPCs from each genotype. As shown in Figure 5g, E2 treatment (10^−8 mol/L) of WT EPCs led to a 35-fold increase in reporter activity over untreated cells. In contrast to WT EPCs, reporter activity was significantly decreased in EPCs from both ERαKO and ERβKO mice (P<0.001 and P<0.003, respectively), indicating that E2-induced, ERE-dependent gene transcription requires both ERα and ERβ.

**E2 Upregulates VEGF Through ERα in EPCs**

To investigate ERα-associated gene expression, we evaluated the repertoire of angiogenic molecules expressed in EPCs by analyzing RNA extracted from WT and ERαKO EPCs. Of
the several angiogenic molecules differentially expressed in WT and ERαKO EPCs, VEGF-A was the most consistent gene expressed differentially, which was confirmed by quantitative RT-PCR (Figure 6a). When WT EPCs were treated with 10^{-8} mol/L E_2, the abundance of VEGF transcripts increased 5-fold within 1 hour of E_2 exposure to WT EPCs (Figure 6b) and persisted for 8 hours (relative expression versus 18S: 26.2±2.4/0 h, 123±12/1 h, 128±21/3 h, 114±14/5 h, and 84±10/8 h), returning to basal levels at 24 hours of E_2 treatment (39.2±1.9/12 h and 28.4±2.0/24 h). In contrast, in ERαKO EPCs, E_2 exposure resulted in a brief, modest increase in VEGF mRNA (relative expression versus 18S: 10.6±0.4/0 h, 24.0±0.2/1 h, 11.9±0.8/3 h, 9.4±0.9/5 h, and 11.0±0.3/8 h). In the mouse MI model, after intravenous...
injection of EPCs just after induction of myocardial injury, we observed that the incorporated WT EPCs, but not ERα/KO EPCs, expressed VEGF (Figure 6c), corroborating the in vitro findings and providing further evidence that ERα-mediated VEGF expression might represent a key feature of the E2-mediated EPC-derived effect on MI recovery.

**Discussion**

Previous studies performed in our laboratory indicated that E2 could enhance the recruitment of EPCs and had favorable effects on neovascularization in ischemic tissues. The focus of the present study was the role of ERs in activation of EPCs during ischemic neovascularization.

These data, from in vitro EPC functional assays, assessment of in vivo EPC homing to ischemic myocardium, and EPC mobilization from bone marrow after MI in mice in which the bone marrow had been replaced with ERα/KO or ERβ/KO mutant marrow, indicate that both ERα and ERβ are functional in EPC-mediated ischemic neovascularization.

The present findings further reveal that the effects of E2 on EPC activation are mediated more prominently via ERα than ERβ. Prior studies of the roles of ERα and ERβ in mediating the macrovascular protective effects of estrogen acting via endothelial cells, including studies in transgenic mice in which either ERα or ERβ expression had been disrupted, have revealed that ERα, not ERβ, mediates the protective effects of estrogen after vascular injury. Our data indicate that certain microvascular effects of E2 involving bone marrow–derived EPCs are mediated predominantly via ERα, but the data also provide evidence of a clear function for ERβ in this setting.

Our data also provide several clues to explain the apparently disproportionate role of ERα in EPC-mediated ischemic neovascularization. First, we found that ERα mRNA expression was 10 times higher than ERβ. In agreement with previous evidence in mature endothelial cells, physiological levels of E2 induce ERα mRNA upregulation in EPCs, indicating that the ligand has potent effects on the expression of its own receptor. Moreover, FITC-conjugated E2-ER binding assay and reporter gene luciferase assay for ERE-dependent transcription support that ERα is a main functional receptor for E2 in EPCs.

Further potential mechanisms of the ERα-specific effects on EPCs were provided by gene expression profiling, which indicated that VEGF was markedly upregulated by E2 in WT but not ERα/KO EPCs. The in vitro findings in isolated EPCs were corroborated in vivo, showing VEGF expression in incorporated WT EPCs in the mouse MI model compared with minimal expression by ERα/KO EPCs. Previous studies have shown that estrogens increase VEGF expression in uterine tissue, endometrial cells, endometrial adenocarcinomas, breast cancer cells, and vascular smooth muscle cells. In cancer cells, E2 was shown to increase VEGF expression.
transcriptional activity through both ERα and ERβ in VEGF promoter luciferase assays via E2–ER complexes binding variant ERE in the promoter region of VEGF gene. Our data indicate that this mechanism is not at play in EPCs.

The actual mechanism by which E2 influences EPC kinetics through ERβ remains unknown. Among 128 angiogenic genes investigated by gene array analysis, we could not find any candidates with consistent and specific expression differences between WT and ERβKO EPCs. A more comprehensive search to identify previously unsuspected candidate genes is under way.

The findings of this report could be of interest for a variety of scientific reasons; however, the impact of our data resides to a large degree on the significant overall effect of estradiol on post-MI outcome. In the present investigation, we concentrated our studies on the mobilization, recruitment, and incorporation of bone marrow–derived cells into vascular structures; however, this paradigm alone may not offer the full explanation for the effect of E2. For example, we did not investigate the possibility that E2 augmented other pathways of tissue repair such as mobilization and homing of cells that can differentiate into cardiomyocytes and stimulation of local cardiac progenitors. In the setting of acute myocardial injury, improved outcome may result via protection (eg, antiapoptosis) and via the full paradigm of tissue repair (ie, replacement of damaged structures). Tissue repair thus requires neovascularization, as documented in the present investigation; however, our studies do not exclude additional E2-mediated effects. Cardiac myocytes and fibroblasts are known to express ER, providing a mechanism for direct effects of E2 on these components of the tissue response to MI.

Finally, beyond the specific findings of this report, these data have important implications because they provide additional evidence of the critical importance of the phenotype of bone marrow–derived EPCs in regulating ischemic tissue repair and suggest that modulation of EPC phenotype may have important therapeutic implications.

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References
CLINICAL PERSPECTIVE

Despite the negative results of recent randomized trials of hormone replacement therapy for the prevention of cardiovascular events, abundant clinical and preclinical data provide evidence of a protective effect of estrogen. For example, premenopausal women are significantly less likely to experience cardiovascular events than are age-matched men, and premature (eg, surgical) menopause is associated with an increased incidence of coronary disease. Most recently, mutations in certain estrogen receptors in humans have been associated with increased incidence of cardiac disease. The present study examined the role of estrogen receptors (ERs) α and β in the improved recovery from myocardial infarction resulting from estradiol (E2) administration. The data reveal that although both ERα and ERβ mediate the effects of E2 on myocardial infarction recovery, ERβ appears to be more critical. These data may lead to the identification of new therapeutic targets for post–myocardial infarction recovery by dissecting the effects of ERα and ERβ. With the knowledge that ERα is more important in the overall benefit of E2, specific downstream signaling pathways that directly mediate the benefit of E2 may be targeted pharmacologically, thereby permitting development of a therapy that mimics the benefits of E2 without the side effects (thrombosis, gynecomasia in men, etc) of the natural or synthetic hormone.
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