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SUPPLEMENTAL MATERIALS AND METHODS

Gene targeting

BAC clones for the periostin gene were isolated from a mouse C57BL6/J BAC library. Targeting strategies are shown in Fig. 2 A. Genomic organization of the *periostin* locus and structure of the targeting vector. The first exon and the PGK-neo cassette are depicted as a closed box and an open box, respectively. The loxP sites are depicted as closed large arrowheads. A diagram of the targeting construct designed to replace the EcoRI–XbaI fragment containing the first exon in the wild-type allele (+) with a PGK-neo cassette is shown, as is the predicted targeted allele (Neo) obtained by homologous recombination. The PGK-neo cassette was then deleted by using the Cre-loxP system. The predicted targeted allele (-) obtained by Cre recombination is also shown. Two homologous fragments of 7.3 kb (XhoI–EcoRI) and 1.2 kb (XbaI–BglII) were subcloned into the PGK-Neo-PGK-DT-A cassette. The linearized vector (50 μg) was electroporated into TT2 ES cells as previously described (1). Among the G418-resistant ES clones examined, 2 clones, #51 and #1051, were detected as homologous recombinants by PCR using a neo-specific primer, PGK-R, and a periostin genomic primer, Peri-R4. The sequences for these primers were 5′-CTA-AAGCGCATGCTCCAGACT-3′ and 5′- GCACCTGCCTCTTCCCAATTACAGG-3′, respectively. These clones were further verified by Southern blot analysis.

Generation of periostin-deficient mice. The aggregation method was used to generate chimeric mice (2). Germline chimeras were obtained by using the ES cell clones #51 and #1051. Chimeric mice with a high contribution of the TT2 genetic background (monitored by agouti coat color) were bred with ICR mice. The #51 chimeric mice were crossed with CAG-Cre mice (3) to excise the neo cassette, and these founder mice were then bred with C57BL/6 mice. For the studies described here, mice carrying periostin (-) alleles were backcrossed to C57BL6/J mice for at least 6 generations.

For genotyping of *periostin*$^{+/+}$ mice and *periostin*$^{-/-}$ mice, PCR with specific primers from within an intron of the periostin gene was used. The following primer pairs were used for amplification of the periostin genomic locus: sense 5′-GTTCTTA-CAGAAAGCAGAAGGATAC-3′ and antisense 5′-TTAAATCACTCCACAGCAGAACACG-3′ for detection of wild-type *periostin*, and sense 5′-CATGATAGCTTCTCTCCCAGTTCTC-3′ and antisense 5′-CTTGCAATAAGTAAAA-CAGCTCCCC-3′ for that of *periostin*$^{-/-}$, as shown in Fig. 2 A (small arrowheads).

Genomic Southern blotting. 10 mg of genomic DNA extracted from normal or targeted ES cell clones was digested with BamHI. The digested genomic DNAs were separated on a 1% agarose gel and transferred onto a Biodyne PLUS membrane (Pall Corporation). Probe labeling and detection of chemiluminescent signals were performed by using a Gene Images Random-Prime Labeling and Detection System (GE Healthcare).

RNA in situ hybridization. Antisense and sense cRNA probes were prepared by in vitro transcription of mouse periostin cDNA (from Fas1 repeat domain 1 to domain 4) and mouse βig-h3 (full-length), by using a DIG Labeling MIX (Roche). Paraffin-embedded, paraformaldehyde-fixed sections (4 μm) were treated with 0.1 M HCL for 20 min, followed by 2×SSC for 5 min. After immersion in proteinase K buffer without enzyme, the sections were digested with 10 mg/ml proteinase K for 15 min, and then treated with 2 mg/ml glycine in PBS. After having been washed with PBS, they were treated with 0.1 M triethanolamine, pH 8.0, for 5 min and then with 0.1 M triethanolamine/0.25% acetic acid anhydride for 10 min. After that, to block endogenous peroxidase activity, we incubated the sections with 3% H_2O_2 for 1 h and washed them with PBS. Next, they were prehybridized for 1 h at room temperature with hybridization buffer composed of 50% formamide, 5×SSC, 10% dextran sulfate, and 250 μg/ml yeast tRNA, and subsequently incubated overnight at 58°C with hybridization buffer containing DIG-labeled antisense or sense probes (200 ng/ml). After hybridization, the sections were rinsed twice in 5×SSC for 15 min at 58°C, and incubated twice (15 min each) in RNase buffer composed of 10 mM Tris-HCl, 500 mM NaCl, and 1 mM EDTA at 37°C. To remove unhybridized RNA, we incubated the sections with 40 μg/ml RNase A in RNase buffer at 37°C for 30 min, and then sequentially washed them, once in RNase buffer at 37°C for 15 min, twice in 0.2×SSC at 58°C for 20 min, and once in 0.2×SSC for 20 min at room temperature. In situ hybridization signals were detected immunohistochemically with horseradish peroxidase–conjugated anti-DIG antibody (Roche) after blocking with 0.5% casein in TBS for 20 min. Signals were visualized with a Tyramide Signal Amplification system (PerkinElmer). The specimens were counterstained with Mayer's hematoxylin, dehydrated, cleared, and coverslipped.

RT-PCR. To determine the expression of periostin in the infarct region after AMI, we purified mRNAs from this region 0, 1, 2, 3, 4, 5, 6, 7, 14, and 28 d after AMI and performed RT-PCR. We also analyzed mRNAs from purified cardiac fibroblasts or purified cardiomyocytes derived from the heart tissue by RT-PCR. For amplification, the following specific primers (shown in Fig. 1 F) were used: primer 1 forward (P1F), 5′-GATAAAATACATCCAAATCAAGTTTGTTCG-3′; primer 1

reverse (P1R), 5′-CGTGGATCACTTCTGTCACCGTTTCGC-3′; P2F, 5′-CTGAAAAACAGACTCGGGAAGAACG-3′; P2R, 5′-AAACTCTGTGGTCTGGCCTCTGGG-3′; P3F, 5′-GATAAAATACATCCAAATCAAGTTTGTTCG-3′; P3R, 5′-AAACTCTGTGGTCTGGCCTCTGGG-3′; periostin (RD1) F, 5′-GGAATTCGGCATTGTGGAGCCAC-TACC-3′; periostin (RD1) R, 5′-GGTCGACTCAAATTTGTGTCAGGACACGGTC-3′; αMHC F, 5′-CTGCTG-GAGAGGTTATTCCTCG-3′; αMHC R, 5′-GGAAGAGTGAGCGGCGCATCAAGG-3′; FSP-1 F, 5′-AGCACTTCCTCTCTCTTGGTCTGGTC-3′; FSP-1 R, 5′-CACTGGCAAACTACACCCCAACAC-3′; GAPDH F, 5′-ACTTTGTCAAGCTCATTTCC-3′; and GAPDH R, 5′-TGCAGCGAACTTTATTGATG-3′.

Ligation of left coronary artery. Induction of AMI was performed as previously described (4). In brief, during anesthesia, 8-wk-old male mice were intubated and connected to a rodent ventilator (SAR-830AP; CWE Inc.). A median thoracotomy was performed, and the left anterior descending artery was identified. Afterward, a 7-0 nylon suture was passed around the artery, and subsequently tied off. Infarction was evident from discoloration of the LV. Finally, the chest wall was closed. Physiological measurements and histological and biological analyses were performed only on surviving mice. Using mid-part sections from at least 5 mice, we determined the infarct size by the previously described method (4).

Echocardiographic and hemodynamic measurements. The echocardiographic system was equipped with an 11 MHz transducer (EnVisor M2540A; Philips). Two-dimensional short-axis views of the LV were obtained. M-mode tracing was recorded through the anterior and posterior LV walls. LV diastolic and systolic internal dimensions (LVESD and LVEDD) were measured. LV percentage of fractional shortening (FS) was calculated as ([LVEDD − LVESD]/LVEDD) × 100.

LV distending pressure/rupture threshold study. *Periostin*$^{+/+}$ and $^{-/-}$ mice were killed 4 d after AMI, and the infarcted hearts were harvested. The rupture threshold stiffness of LVs was determined as previously described (5).

Myeloperoxidase assay. A myeloperoxidase assay, which is an index of neutrophil infiltration in the infarct hearts, was performed as previously described (6). Infarcted hearts were weighed and frozen at −70°C, and 50 mg of the frozen tissue was sequentially homogenized in 1 ml of HTAB buffer (0.5% of HTAB and 50 mM Na_2HPO_4, pH 5.4), sonicated, subjected to 3 rounds of freezing/thawing, and then centrifuged at 20,000 g for 5 min. 10 μl of supernatant was transferred into a flat-bottomed 96-well plate, and 200 μl of *O*-dianisidine hydrochloride solution (1.76 mg/ml of *O*-dianisidine in 50 mM Na_2HPO_4, pH 5.4) was added immediately. After incubation, the absorbance was measured at 450 nm.

Cell culture. Primary adult ventricular cardiac fibroblasts were isolated from *periostin*$^{+/+}$ and $^{-/-}$ mice and maintained as previously described (7). C3H10T1/2 cells were grown in DME supplemented with 10% FNS (Invitrogen). Cells were transfected with siRNA oligonucleotides at 50 nM (SMARTpool; Dharmacon) by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The accession numbers of SMART pool siRNA oligonucleotides were as follows: L-041099-00 for *fak* and L-046779-01 for *αv-integrin*. SH-6 (10 μM; an Akt inhibitor), PP2 (10 μM; a Src and FAK tyrosine kinase inhibitor; Calbiochem), Akt 1/2 inhibitor (10 μM; Sigma-Aldrich), or Geneticin (10 μM; a tyrosine kinase inhibitor; Fujicco) was added 30 min before stimulation.

Western blotting. PVDF membranes were probed with antibodies against phosphor-Akt (Ser 437), total Akt (Cell Signaling Technology), phosphor-FAK (Tyr 397; Biosource), total FAK (BD Biosciences), β-actin (Sigma-Aldrich), or mouse periostin (our laboratory).

In vitro cell migration assay. Cardiac fibroblasts from 8-wk-old *periostin*$^{-/-}$ mice were harvested and washed twice in serum-free DME containing 0.1% BSA (DME-BSA). Final volumes were adjusted for all cell preparations to be 1.0×10^5 cells/ml. 2.5×10^4 cells were added to each upper chamber of a 24-well transwell chamber (BD Biosciences) and allowed to migrate from DME-BSA– (upper chambers) to DMEM-BSA–containing medium conditioned by periostin ΔbΔe- or empty vector-transfected HEK293T cells (lower chambers). As inhibiting molecules, 10 μg/ml anti-periostin (R&D Systems) and 10 μg/ml anti–αv-integrin (CHEMICON Intenational, Inc.) antibodies, 10 μM PP2, or 50 nM siRNAs were used. After incubation for 24 h, migrating cells were fixed and stained with Giemsa solution. The net number of cells that had migrated completely through the 8-μm pores was determined in 10 random high-power fields (400×) for each filter. The migration assays were performed in triplicate.

TGFβ-neutralizing antibody treatment. Wild-type male mice received intraperitoneal injections of TGFβ-neutralizing antibody (R&D Systems) once every 7 d starting at 7 wk of age. Both TGFβ 1 and 2 are known to be neutralized in vivo and in vitro by this antibody (8). We diluted the antibody in PBS and administered it at a dose of 5 mg/kg body weight. Rabbit IgG (5 mg/kg; Sigma-Aldrich) was used in a similar fashion as a negative control. Induction of AMI was performed at 8 wk of age. Mice were killed 5 d after AMI, and we used 5 mice per group for histological analysis.

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Table S1. Echocardiographic data

	Control		MI day 1		MI day 7		MI day 28	
	periostin$^{+/+}$	*periostin*$^{-/-}$	*periostin*$^{+/+}$	*periostin*$^{-/-}$	*periostin*$^{+/+}$	*periostin*$^{-/-}$	*periostin*$^{+/+}$	*periostin*$^{-/-}$
BW (g)	20.4 ± 0.3	20.3 ± 0.3	20.4 ± 0.5	20.4 ± 0.6	20.1 ± 0.3	19.7 ± 0.4	24.3 ± 0.4	24.3 ± 0.5
HR (beats/min)	458 ± 14	461 ± 9	425 ± 14	415 ± 14	397 ± 23	408 ± 15	458 ± 12	461 ± 21
LVEDD (mm)	3.44 ± 0.05	3.41 ± 0.03	3.82 ± 0.06	3.82 ± 0.06	4.66 ± 0.19	4.15 ± 0.03[a]	5.19 ± 0.17	4.48 ± 0.23[a]
LVESD (mm)	1.76 ± 0.03	1.73 ± 0.02	2.88 ± 0.08	2.88 ± 0.08	3.66 ± 0.24	3.09 ± 0.04[a]	4.47 ± 0.20	3.53 ± 0.29[a]
AW (mm)	0.59 ± 0.01	0.58 ± 0.00	0.59 ± 0.03	0.59 ± 0.04	0.49 ± 0.01	0.50 ± 0.01	0.45 ± 0.01	0.48 ± 0.02
PW (mm)	0.60 ± 0.01	0.58 ± 0.00	0.58 ± 0.01	0.57 ± 0.01	0.61 ± 0.01	0.59 ± 0.03	0.66 ± 0.02	0.63 ± 0.02
FS (%)	48.9 ± 0.6	49.2 ± 0.3	24.7 ± 1.0	24.6 ± 1.5	21.9 ± 2.4	25.6 ± 0.9	14.3 ± 1.5	22.1 ± 2.9[a]
Infarct size (%)	–	–	50.9 ± 2.4	51.1 ± 3.7	50.1 ± 2.7	49.4 ± 3.3	46.8 ± 2.1	45.5 ± 2.4

Echocardiographic parameters in the *periostin*$^{+/+}$ and $^{-/-}$ mice under basal conditions and at 1, 7, or 28 d after LAD ligation. We demonstrated that in the case of periostin deficiency, the collagen amount was reduced in the infarct myocardium, resulting in frequent cardiac rupture in the acute phase, whereas failure in dilation and preservation of cardiac function occurred in the chronic phase in the MI model. In contrast, in the chronic phase of MI, loss of periostin resulted in improvement of cardiac function. Our echocardiographic data of the chronic phase of MI are consistent with the results reported by Oka et al. (Oka, T., J. Xu, R.A. Kaiser, J. Melendez, M. Hambleton, M.A. Sargent, A. Lorts, E.W. Brunskill, G.W. Dorn, II, S.J. Conway, B.J. Aronow, J. Robbins, and J.D. Molkentin. 2007. *Circ. Res.* 101:313–321). Their studies, however, focused on analysis of the cardiac hypertrophy and ventricular remodeling in the chronic phase of MI, but it provided no mechanistic insight into the increase in cardiac rupture in *periostin*$^{-/-}$ mice. BW, body weight; HR, heart rate; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; AW, anterior wall thickness; PW, posterior wall thickness; FS, percentage of fractional shortening.

[a]$P < 0.05$ versus *periostin*$^{+/+}$ with AMI.