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Fibroblast Growth Factor-2 Regulates Myocardial Infarct Repair

Effects on Cell Proliferation, Scar Contraction, and Ventricular Function

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Fibroblast growth factor-2 (FGF2, bFGF) has been proposed to regulate wound healing and angiogenesis, but skin wound healing in FGF2-knockout (FGF2-KO) animals is only slightly delayed. To determine the role of FGF2 in myocardial infarct repair, we studied the evolution of left ventricular geometry, cell proliferation, matrix content, and cardiac function in mice lacking or overexpressing (FGF2-Tg) FGF2. Despite having no effect on initial infarct size, deletion of FGF2 resulted in reduced fibroblast proliferation and interstitial collagen deposition, decreased endothelial proliferation and vascular density, and decreased cardiomyocyte hypertrophy. Furthermore, FGF2-KO mice demonstrated a complete absence of scar contraction, resulting in increased final infarct size and marked increases in chamber size and infarct expansion. These deficits ultimately impaired left ventricular dP/dt compared with wild-type infarcted mice. Conversely, overexpression of FGF2 increased fibroblast proliferation and collagen deposition, accelerated endothelial proliferation, and enhanced cardiomyocyte hypertrophy after infarction. These changes curbed infarct expansion and preserved left ventricular function. Thus, FGF2 is an important regulator of cell proliferation, angiogenesis, collagen synthesis, myocyte hypertrophy, scar contraction, and, ultimately, left ventricular contractile function during infarct repair. FGF2 may be more important in healing of infarcts compared with skin wounds because of the mechanical stress under which infarcts heal. ***(Am J Pathol 2007, 171:1431–1440; DOI: 10.2353/ajpath.2007.070003)***

Myocardial infarction results in the loss of irreplaceable contractile elements. Necrotic tissue is removed by macrophages and replaced with granulation tissue, which is eventually transformed into a collagenous scar. During this process the infarcted wall thins, the left ventricular (LV) chamber dilates, and there is interstitial fibrosis and cardiomyocyte hypertrophy in the noninfarcted region of the ventricle. These changes are associated with cardiac dysfunction and progression to heart failure. Based on the observation that late reperfusion (too late to salvage myocardium) both increases the rate of wound healing and attenuates ventricular dilation,[1] we have proposed that identifying growth factors involved in infarct repair could provide additional targets to prevent ventricular dysfunction.[2] We previously studied baseline variables of repair in a murine model of myocardial infarction induced by permanent coronary artery ligation. We found that fibroblast and endothelial cell proliferation peak at 4 days, that vascular density decreases as the infarct heals (although the total vessel area remains constant), and infarct scars contract to ~50% of the original infarct size.[2] These baseline measurements provide a means to understand the factors that govern the process of infarct repair and for investigating therapeutic molecules that promote healing.

Fibroblast growth factor-2 (FGF2, bFGF) has long been known to stimulate proliferation of cultured mesenchymal

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cells such as fibroblasts, endothelial cells, smooth muscle cells, and skeletal myoblasts, and it is also involved in regulation of cell survival, migration, and matrix production/degradation.[3] Furthermore, exogenous administration of FGF2 causes angiogenesis and fibroblast proliferation *in vivo*.[4] These properties led to a widespread hypothesis that FGF2 was an important regulator of tissue repair. Surprisingly, however, FGF2-KO mice were viable, fertile, and showed only a modest delay in closure of excisional skin wounds,[5] suggesting FGF2 was not a major player in repair of the skin. In the heart, however, Schultz and colleagues[6] reported that FGF2-KO mice exhibited reduced cardiomyocyte hypertrophy and interstitial fibrosis after aortic banding. Because fibrosis and hypertrophy are important components of the heart's response to infarction, this suggested that FGF2 might be an important regulator of myocardial infarct repair.

In the present study, we have used FGF2-KO and overexpressing (FGF2-Tg) mice to determine the role of FGF2 in myocardial infarct repair. We hypothesized that the absence of FGF2 would lead to decreased proliferation of fibroblasts and endothelial cells, resulting in ventricular dilation and impaired function. Conversely, we theorized that overexpression of FGF2 would augment cell proliferation and thereby reduce dilation and ameliorate cardiac dysfunction.

Materials and Methods

Animals

Male mice, 6 to 8 weeks of age, were used in this study. Wild-type (WT) C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). FGF2-KO mice were obtained from Dr. Dan Bowen-Pope, Department of Pathology, University of Washington, Seattle, WA, also on a C57BL/6J background. Briefly, FGF2-KO mice were produced by replacing 0.5 kb (including the first exon and part of the proximal promoter) of the FGF2 gene with an Hprt minigene, resulting in deletion of both the high- and low-molecular weight isoforms.[7] FGF2-Tg mice (FVB/N background) and WT controls were obtained from Dr. Doug Coffin, University of Montana, Missoula, MT.[7,8] In the FGF2-Tg mice, the human full-length FGF2 cDNA, including all three translational start sites, is ubiquitously expressed under the control of the phosphoglycerate kinase promoter. Nontransgenic FVB/N littermates (FGF2-nTg) were used as controls for the transgenic animals. The procedures for the care and treatment of mice were followed according to those set by the University of Washington Animal Care and Use Committee guidelines. Western blotting demonstrated that transgenic mice had threefold to sevenfold overexpression of FGF2 in the LV myocardium (Supplemental Figure 1 at *http://ajp.amjpathol.org*). Attempts to extract FGF2 from the infarcted heart were unsuccessful because of low protein yield.

Surgical Procedure

Male mice (20 to 30 g) were anesthetized with an intraperitoneal injection of 20 μl/g body weight Avertin (20 mg/ml). The surgical procedure has been described in detail elsewhere.[9,10] Briefly, the mouse was mechanically ventilated, a thoracotomy was performed, and the left anterior descending coronary artery was ligated using a dissecting microscope and a fiber optic light source. Control animals underwent the same procedure without ligation. The animals were permitted to recover in a heated chamber before being returned to the vivarium. Survival rates were as follows: WT, 92%; FGF2-KO, 86%; FGF2-nTg, 94%; and FGF2-Tg, 91%.

At 2 days, 4 days, 1 week, 2 weeks, and 4 weeks, most mice were given a 0.5-ml intraperitoneal injection of 5-bromodeoxyuridine (BrdU; 5 mg/ml) to label proliferating cells and sacrificed 1 hour later with an intraperitoneal injection of pentobarbital. In animals in which a cumulative labeling of proliferating cells was determined, miniosmotic pumps (Alzet model 1007D, elution rate: 0.5 μl/hour; Durect Corp., Cupertino, CA) were filled with BrdU (62.5 μg/μl in 50:50 dimethyl sulfoxide/H_2O; total volume 90 μl) and placed in the dorsal subcutaneous space immediately after the infarct surgery. The heart and small intestine (as a proliferation control) were briefly rinsed in phosphate-buffered saline (PBS) and immersed in zinc fixative as previously described.[9] After fixation, the heart was transversely sectioned into four slices of equal thickness, processed, and embedded in paraffin by routine methods. Routine histological procedures [hematoxylin and eosin (H&E) and picrosirius red/fast green staining] and immunostaining were performed using 5-μm sections.

Morphometry and Histology

Photographs of four H&E-stained sections per heart were taken at $\times$20 using a SPOT RT digital camera and the SPOT imaging program (Diagnostic Instruments, Sterling Heights, MI). The images were randomized and the investigator was blinded. Scion imaging software (Scion Corp., Frederick, MD) was calibrated with a micrometer (Olympus, Melville, NY) and used to trace the cross-sectional area of the LV wall and chamber, infarct zone, necrosis, granulation tissue, scar, and to measure scar and septal wall thickness. The measurements were exported into Excel for analysis. Expansion index was calculated as the septal thickness/scar thickness $\times$ chamber area/LV area.[11]

To assess myocyte cross-sectional area, five pictures were taken at $\times$600 from both the epicardial and endocardial surfaces in two sections from each heart, for a total of 10 pictures per heart (three hearts/time point). In each picture, the diameter of three to seven myocytes sectioned through the short axis was measured at the level of the nucleus, and the mean cross-sectional area was calculated.

To quantify interstitial fibrosis, slides were stained with picrosirius red for fibrillar collagen, and the cytoplasm was counterstained with fast green for contrast.[12] Five images at $\times$400 were taken in the posteroseptal noninfarcted region. Using Adobe Photoshop software (Adobe Systems, Mountain View, CA), the red collagen fibril pixels were counted and expressed as a percentage of the total number of pixels (total = green + red − white).

Vessel number and area were determined in four images (two epicardial, two endocardial; ×400) of the infarct zones of control, 1-week, and 4-week hearts stained with an anti-CD31 antibody. The number of vessels in each field was counted, the vessel areas were measured, and the analysis was performed in Excel. The data were expressed as average number of vessels/mm^2, the average vessel area as a percentage of total tissue area, and the average area per vessel.

Immunostaining

Tissue sections were deparaffinized in 2 × 5 minutes xylene rinses followed by 2 × 5 minutes 100% ethanol rinses. Endogenous peroxidases were quenched in 0.3% H_2O_2 in methanol for 20 minutes. After rinsing 3 × 5 minutes in PBS, slides were incubated overnight at 4°C with either anti-smooth muscle α-actin (peroxidase-conjugated mouse anti-human monoclonal, U7033; DAKO, Carpinteria, CA) for myofibroblasts[2,13] or anti-CD31 (rat anti-mouse monoclonal, no. 553371, 1:2000; PharMingen, La Jolla, CA) for endothelial cells.[2,9] The reaction product was visualized with the chromogen diaminobenzidine (SK-4100; Vector Laboratories, Burlingame, CA). For BrdU double-labeling of fibroblasts or endothelial cells, slides previously stained with anti-smooth muscle α-actin or anti-CD31, respectively, were immersed in 1.5 N HCl at 37°C for 15 minutes, briefly rinsed in distilled water, placed in 2 × 5 minutes borax buffer (pH 8.5), rinsed in PBS, and incubated with peroxidase-conjugated anti-BrdU (no. 1585860, 1:25; Roche, Indianapolis, IN) overnight at 4°C. The reaction product was visualized with Vector VIP (SK-4600; Vector). All slides were subsequently counterstained with methyl green, dehydrated, and coverslipped. Proliferating cells were counted using a 10 × 10-grid reticle eyepiece by surveying random fields from the border zone of one end of the infarct to the other until a total of 500 cells had been counted. Fibroblasts were counted at ×400, and endothelial cells were counted at ×600. Measurements were expressed as the percentage of double-labeled cells in 500 diaminobenzidine-positive cells ± SEM.

LV Function

Four weeks after myocardial infarction, mice were heparinized (0.94 U/g i.p.) and anesthetized with an intraperitoneal cocktail containing ketamine (115 μg/g), xylazine (3.5 μg/g), and buprenorphine (0.35 μg/g). Body temperature was regulated using a rectal probe in conjunction with a temperature controller (Harvard 7129; Harvard Apparatus, Holliston, MA). A tracheotomy was performed and mice were ventilated (Harvard 687, Harvard Apparatus) such that expiratory CO_2 was maintained between 2.8 and 3.2% (microcapnometer; Columbus Instruments, Columbus, OH). A 1.4-French catheter (Millar, Houston, TX) was advanced into the ventricle via the right carotid artery, and LV pressure and electrocardiogram were recorded.[14] The first derivative of the LV pressure curve, dP/dt, was calculated (DataQ software), and the +dP/dt at 20 mm Hg above end-diastolic pressure was used to compare contractility among experimental groups. Of the 87 mice that were attempted to be analyzed, 63 valid traces were obtained and 24 mice were lost because of bleeding or unsuccessful catheterization.

Statistics

All group data are expressed as mean ± SEM. Statistical significance between groups was determined by Student's *t*-tests or analysis of variance followed by Student-Newman-Keuls multiple comparison post hoc analyses. All statistics were performed using Instat, and significance levels were $P < 0.05$.

Results

Ventricular Geometry

Figure 1 shows representative H&E-stained sections of noninfarcted hearts (control; Figure 1, A, D, G, and J), 4-day infarcts (Figure 1, B, E, H, and K), and 4-week infarcts (Figure 1, C, F, I, and L) from WT, FGF2-KO, FGF2-nTg, and FGF2-Tg mice, respectively. These micrographs depict the morphological changes that mouse hearts undergo after MI—ventricular wall thinning, chamber dilation, and the transition from necrosis (N) encapsulated by granulation tissue at 4 days to mature scar (S) by 4 weeks. Hearts of noninfarcted control mice from all groups exhibited comparable morphology and geometry.

Table 1 summarizes the morphometric data for WT versus FGF2-KO mouse hearts. Initial infarct size at 4 days after MI was 38 ± 5% in the WT and 37 ± 4% in the FGF2-KO group (P = ns). In WT mouse hearts, infarct size decreased substantially because of scar contraction, reaching approximately one-half the original infarct size at 4 weeks (Figure 2E). Despite having similar initial infarct size, the FGF2-KO hearts did not undergo scar contraction throughout the 4-week study. As might be anticipated from this lack of wound contraction, LV dilation was much more severe in the FGF2-KO hearts. For example, in WT C57BL/6 animals, chamber area was increased to sixfold at 4 weeks, whereas in FGF2-KO hearts chamber area was increased 10-fold at 4 weeks (P = 0.004 versus WT 4-week infarct). Although there were no significant differences in infarct composition (ie, necrosis, granulation tissue, or scar as a percentage of the infarct) at any of the time points studied, in FGF2-KO hearts there was a tendency for less granulation tissue formation at 4 days and 1 week and more persistent residual necrosis (throughout 4 weeks). Although scar thickness was not different between the groups, expansion index in the FGF2-KO mice was twice that of the WT mice at 4 weeks (Figure 2A; 3.11 ± 0.77 versus 1.49 ± 0.26), indicating more severe LV remodeling.

Table 2 summarizes the morphometric data for FGF2-nTg versus FGF2-Tg mice. Infarct size in FGF2-nTg mouse hearts (WT FVB/N strain) 4 days after MI was 34 ± 6% of the LV and contracted to 16 ± 4% at 4 weeks. In mice overexpressing FGF2, there was a nonsignificant

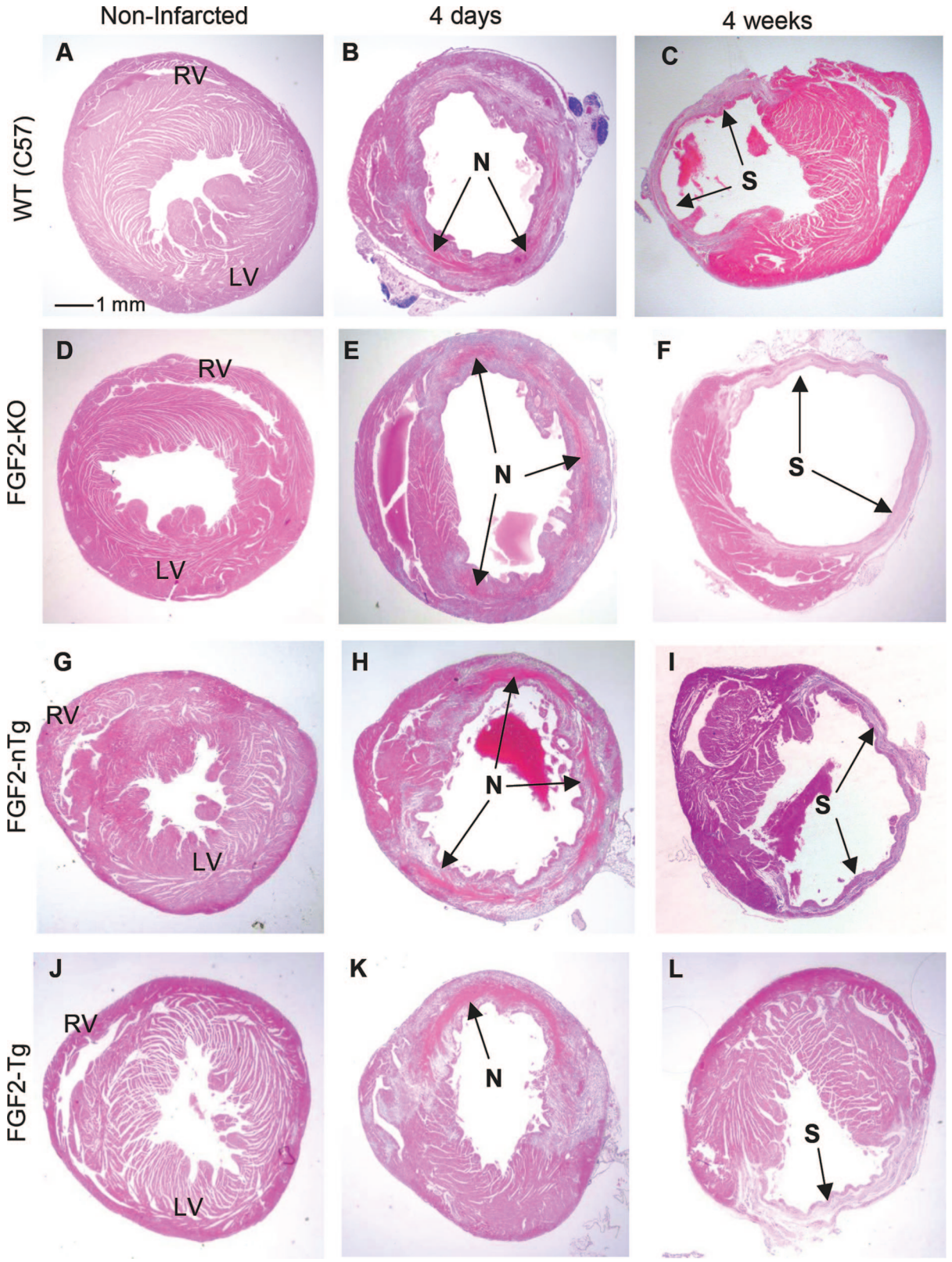

Figure 1. Histology of infarct repair in FGF2-KO and FGF2-Tg mice. Noninfarcted hearts of all groups studied (**A**, **D**, **G**, and **J**) exhibited uniform thickness of the left (LV) and right (RV) ventricular walls, comparable chamber area, and normal myocyte morphology. Four days after MI (**B**, **E**, **H**, and **K**), hearts of all groups had residual necrosis (N) surrounded by granulation tissue. In all groups studied at 4 weeks after MI (**C**, **F**, **I**, and **L**), there was thinning of the ventricular wall, chamber dilation, and collagenous scar (S) formed to replace the dead myocardium. The greatest chamber dilatation and ventricular wall thinning occurred in the FGF2-KO at 4 weeks after MI. The least extensive wall thinning occurred in the FGF2-Tg hearts. Scale bar = 1 mm.

Table 1. Left Ventricular Morphometry in WT Versus FGF2-KO Mouse Hearts

	Group	Control	4 days	1 week	2 weeks	4 weeks
Group size	WT	5	12	9	7	8
	FGF2-KO	5	14	10	8	8
LV area (mm^2)	WT	2.52 ± 0.21	2.24 ± 0.20	1.83 ± 0.17	2.03 ± 0.26	2.63 ± 0.21
	FGF2-KO	2.51 ± 0.31	2.41 ± 0.17	2.19 ± 0.14	1.92 ± 0.25	2.38 ± 0.3
Chamber area (mm^2)	WT	0.16 ± 0.06	0.73 ± 0.13	0.49 ± 0.05	0.74 ± 0.11	0.96 ± 0.14
	FGF2-KO	0.18 ± 0.03	0.90 ± 0.10	0.83 ± 0.10*	1.57 ± 0.21*	1.79 ± 0.22*
Infarct size (% LV)	WT		37.5 ± 5.3%	26.8 ± 4.4%	31.8 ± 5.2%	19.7 ± 3.5%
	FGF2-KO		37.3 ± 3.9%	40.7 ± 3.9%*	56 ± 7.7%*	44.3 ± 6.8%*
Granulation tissue (% infarct)	WT		72.7 ± 2.4%	95.3 ± 1.2%	0	0
	FGF2-KO		69 ± 2.4%	89.8 ± 2.4%	0	0
Necrosis (% infarct)	WT		27.3 ± 2.4%	4.7 ± 1.2%	0	0
	FGF2-KO		31 ± 2.6%	10.2 ± 2.4%	3.6 ± 2.1%	2.8 ± 2.6%
Scar (% infarct)	WT		0	0	100%	100%
	FGF2-KO		0	0	96.4 ± 2.1%	97.2 ± 2.6%
Scar thickness (mm)	WT					0.16 ± 0.02
	FGF2-KO					0.16 ± 0.02

*$P < 0.05$ versus WT.

tendency for the scar to reduce further (30 ± 5% at 4 days versus 11 ± 3% at 4 weeks). The FVB/N mice showed less ventricular dilation than did the C57BL/6 mice, with chamber size increasing only twofold at 4 weeks. Although chamber size was slightly smaller in the FGF2-Tg mice at 4 days after MI (0.42 ± 0.15 versus 0.55 ± 0.22 mm^2, $P = 0.04$), dilation at 4 weeks was not significantly different between genotypes. Scar thickness in FGF2-Tg mice was twice that of the FGF2-nTg mice at 4 weeks (0.64 ± 0.07 versus 0.30 ± 0.04 mm, $P < 0.05$), resulting in the observed difference in expansion index (Figure 2B, 0.77 ± 0.13 versus 1.91 ± 0.15).

Myocyte cross-sectional area was measured to determine whether FGF2-KO myocytes have an intact hypertrophic response after myocardial infarction compared with WT. Myocyte cross-sectional area in the WT 4-week infarcts increased by 19% ($P < 0.05$ versus noninfarcted control). In infarcted FGF2-KO hearts, however, there was a negligible increase of only 4% (versus noninfarcted control, Figure 2C). Conversely, FGF2-Tg mice showed increased cardiomyocyte hypertrophy after infarction. Myocyte cross-sectional area increased 19% in FGF2-nTg hearts ($P < 0.01$) compared with 47% in the FGF2-Tg mouse hearts ($P < 0.001$, Figure 2D).

Fibroblast Proliferation and Fibrosis

Proliferating myofibroblasts were labeled with smooth muscle α-actin and BrdU antibodies (Supplemental Figure 2 at *http://ajp.amjpathol.org*). There was no significant proliferation of any cell type in WT C57BL/6 hearts or FGF2-KO hearts 2 days after MI (Figure 3A). All groups showed a burst of fibroblast proliferation at 4 days, which slowed by 1 week, and ceased by 2 weeks. In the FGF2-KO mice, fibroblast proliferation was 33% lower at 4 days (10.6 ± 1.1% versus 15.4 ± 1.1%, $P < 0.01$) and 59% lower at 1 week (1.7 ± 0.2% versus 4.1 ± 0.6%, $P < 0.05$) than in WT mice. In hearts of mice overexpressing FGF2, fibroblast proliferation (Figure 3B) was more than twice that of WT hearts at 2 days (2.5 ± 1% versus 1.0 ± 0.4%, $P < 0.05$), at 4 days (7.5 ± 1% versus 3.8 ± 0.5%, $P < 0.05$), and also at 1 week (1.7 ± 0.5% versus 0.8 ± 0.2%, $P < 0.05$). It is worth noting that cardiac fibroblast proliferation rates in WT FVB/N mice were lower than in WT C57BL/6 mice, although the total duration of fibroblast proliferation was longer.

Because fibroblasts are the cell type responsible for collagen deposition, we quantified interstitial fibrosis in the remote, noninfarcted region and correlated it with the observed differences in fibroblast proliferation. Representative sections of noninfarcted control hearts (left column) and 4-week-old infarcted hearts (right column) were stained with picrosirius red/fast green to illustrate collagen deposition (Supplemental Figure 3 at *http://ajp.amjpathol.org*). Interstitial fibrosis increased 167% in WT infarcts at 4 weeks (0.9 ± 0.1% versus 2.4 ± 0.4% of myocardial area, $P < 0.05$) compared with WT noninfarcted controls (Figure 3C), but did not increase after infarction in FGF2-KO (1.2 ± 0.4% in control versus 1.3 ± 0.1% in infarcted, P = ns). Transgenic overexpression of FGF2 increased interstitial collagen content by 75% in noninfarcted hearts. In FGF2-nTg hearts, infarction resulted in a 58% increase in fibrosis compared with control hearts (1.2 ± 0.1% versus 1.9 ± 0.4%, P = ns; Figure 3D). Hearts overexpressing FGF2 showed an exaggerated fibrotic response after infarction, with collagen content increasing by 81% (2.1 ± 0.5% versus 3.8 ± 0.8%, $P < 0.05$) compared with noninfarcted controls. These results suggest that decreased fibroblast proliferation led to a reduction in collagen deposition in FGF2-KO mice; conversely, in the overexpressing mice, increased fibroblast proliferation because of injury resulted in increased interstitial fibrosis.

Endothelial Cell Proliferation, Vascular Morphometry

Endothelial cells were double-labeled with CD31 and BrdU antibodies to quantify their proliferation (Supplemental Figure 2 at *http://ajp.amjpathol.org*). Pulse-labeling studies showed that endothelial cell proliferation also peaked at 4 days after MI in both WT and FGF2-KO

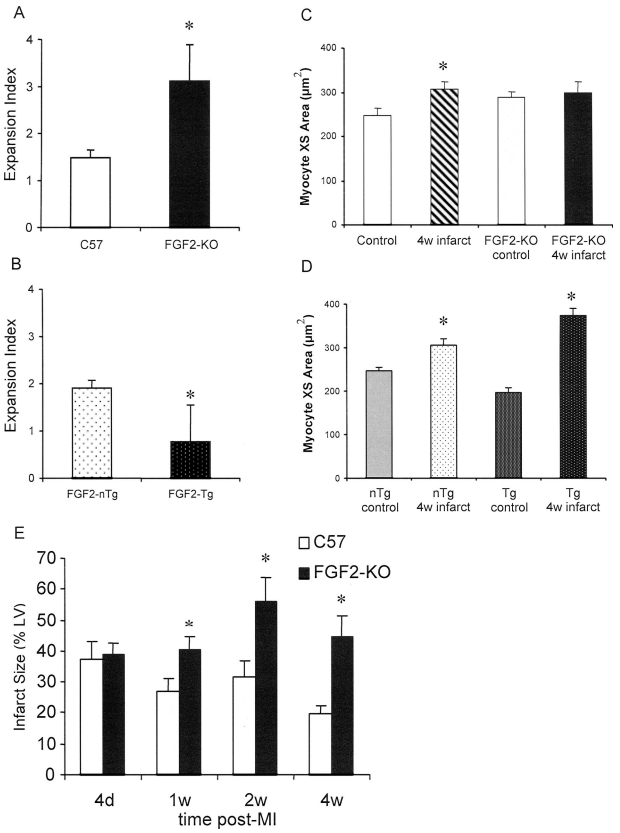

Figure 2. Postinfarct changes in LV geometry and cardiomyocyte hypertrophy. Group sizes ranged from 7 to 14 mice per time point, as listed in Tables 1 and 2. **A:** Four weeks after MI, the expansion index [(septal thickness/scar thickness) × (chamber area/LV area)] of FGF2-KO mice was twice that of WT mice ($P = 0.03$). **B:** Conversely, the expansion index in the FGF2-Tg group was half that of FGF2-nTg mouse hearts ($P = 0.0006$). **C:** Myocytes of WT mice exhibited a significant hypertrophic response as evidenced by an increase in myocyte cross-sectional area (XS-area, $P = 0.02$) whereas FGF2-KO mice showed no significant hypertrophy ($P = 0.67$). **D:** Although myocyte cross-sectional area was smaller in noninfarcted FGF2-Tg hearts than in FGF2-nTg hearts ($P = 0.001$), the hypertrophic response in the FGF2-Tg group ($P < 0.001$) was more than twice that observed in FGF2-nTg mouse hearts ($P = 0.001$). Each column represents ~100 myocytes per heart (three to eight hearts per group). **E:** Infarcts in WT C57BL/6 mice underwent the expected wound contraction to ~50% their initial size by 4 weeks. In contrast, infarcts from FGF2-KO mice failed to undergo wound contraction, such that they were twice as large as observed WT mice at 4 weeks. *$P < 0.05$ FGF2-KO versus C57 WT.

hearts. The rate of endothelial proliferation was much lower than observed for fibroblasts, however, making statistical analysis more difficult using simple BrdU pulse labeling. We therefore determined cumulative endothelial cell proliferation by infusing BrdU for 4 days via osmotic minipumps. This revealed that the cumulative rate of proliferation 4 days after ligation was 65% less in FGF2-KO hearts (1.9 ± 0.5% versus 5.4 ± 1.5%, $P < 0.05$) than in WT hearts (Figure 4A).

In hearts of mice overexpressing FGF2, significantly increased endothelial proliferation was detected by pulse-labeling, and hence, cumulative labeling was not performed. At 2 days after MI, endothelial cell proliferation in FGF2-Tg mouse hearts was threefold greater than that of the FGF2-nTg hearts (Figure 4B, $P < 0.05$). At 4 days after MI, endothelial proliferation in FGF2-Tg hearts was 1.7-fold greater than FGF2-nTg hearts. There was no significant endothelial proliferation observed in noninfarcted control hearts or beyond 1 week after MI in any of the groups studied.

To correlate observed changes in endothelial cell proliferation with changes in vascular density, we counted vessels, quantified vessel area, and calculated the average area/vessel. Vascular density was comparable in noninfarcted WT and noninfarcted FGF2-KO mouse hearts (3644 ± 82 versus 2963 ± 227 vessels/mm^2, respectively; Figure 4C). Vascular density declined significantly after infarction in both WT and FGF2-KO hearts, but this decline was more dramatic in the FGF2-KO group: 74 versus 25% at 1 week and 91 versus 75% at 4 weeks after MI ($P < 0.01$ at both times). Interestingly, there was no difference in the vessel area (expressed as a percentage of the total myocardial area) between all six groups analyzed (Figure 4E). The discrepancy between the decreased number of vessels/mm^2 and no change in the vessel area in the 4-week infarcts of FGF2-KO mice was explained by the calculated 10-fold increase in area/vessel (control: WT, 13.4 ± 0.4 μm^2; FGF2-KO, 12.8 ± 0.5 μm^2; 4-week infarct: WT, 38.7 ± 7.9 μm^2; FGF2-KO, 122.9 ± 23.4 μm^2; $P < 0.001$) (Figure 4E). These large sinusoidal vessels often lacked smooth muscle/pericyte investment. Although endothelial cell proliferation peaked earlier in FGF2-Tg compared with FGF2-nTg mouse hearts ($P < 0.05$), no significant changes were observed in vascular density, vascular area or area/vessel, or vessel muscularity (Figure 4, F–H).

LV Function

A 1.4-French Millar catheter introduced into the left ventricle via the carotid artery was used to measure LV pressure in noninfarcted control mouse hearts and hearts 4 weeks after MI. The first derivative of LV pressure with respect to time (dP/dt; mm Hg/second) at 20 mm Hg was measured to reveal differences in contractility (Figure 5; full data set in Supplemental Table 1, see *http://ajp.amjpathol.org*). In WT mice, there was an 11% decrease in dP/dt at 4 weeks after MI versus control (4807 ± 333 versus 4247 ± 188 mm Hg/second; P = ns) compared with a 20% reduction in FGF2-KO hearts 4 weeks after MI versus noninfarcted FGF2-KO hearts (5107 ± 245 versus 4110 ± 339 mm Hg/second; $P = 0.02$) (Figure 5A). In FGF2-nTg mice, LV dP/dt decreased by 20% (5194 ± 134 versus 4171 ± 171 mm Hg/second, $P = 0.04$) compared with only 12% in the FGF2-Tg group (4459 ± 377 versus 3906 ± 129 mm Hg/second; P = ns) (Figure 5B). Although there were strain differences in heart rate, there were no significant differences in HR within the experimental groups (Figure 5, A and B).

Discussion

We have shown that genetic deletion of FGF2 markedly worsened the process of myocardial infarct repair. Despite having no effect on initial infarct size, deletion of FGF2 resulted in reduced fibroblast proliferation and collagen deposition, decreased endothelial proliferation and vascu-

Table 2. Left Ventricular Morphometry in FGF2-nTg Versus FGF2-Tg Mouse Hearts

	Group	Control	4 days	1 week	2 weeks	4 weeks
Group size	FGF2-nTg	7	8	6	8	8
	FGF2-Tg	4	9	7	7	8
LV area (mm^2)	FGF2-nTg	2.37 ± 0.43	1.90 ± 0.40	2.17 ± 0.66	1.99 ± 0.58	2.42 ± 0.67
	FGF2-Tg	2.17 ± 0.49	1.86 ± 0.44	2.17 ± 0.56	2.00 ± 0.62	2.33 ± 0.57
Chamber area (mm^2)	FGF2-nTg	0.35 ± 0.13	0.55 ± 0.22	0.48 ± 0.27	0.59 ± 0.27	0.63 ± 0.25
	FGF2-Tg	0.29 ± 0.16	0.42 ± 0.15	0.46 ± 0.19	0.53 ± 0.17	0.51 ± 0.20
Infarct size (% LV)	FGF2-nTg		34.4 ± 6.3%	19.3 ± 4.8%	17.4 ± 5.1%	16.3 ± 3.7%
	FGF2-Tg		30.1 ± 5.1%	16.6 ± 4.9%	20.9 ± 5.7%	11.3 ± 2.9%
Granulation tissue (% infarct)	FGF2-nTg		72.9 ± 3%	94.2 ± 2.1%	0	0
	FGF2-Tg		80.7 ± 2.9%	98.3 ± 1.7%	0	0
Necrosis (% infarct)	FGF2-nTg		27.1 ± 2.6%	5.8 ± 1.9%	0	0
	FGF2-Tg		19.3 ± 2.8%	1.7 ± 1.6%	0	0
Scar (% infarct)	FGF2-nTg		0	0	100%	100%
	FGF2-Tg		0	0	100%	100%
Scar thickness (mm)	FGF2-nTg					0.30 ± 0.04
	FGF2-Tg					0.64 ± 0.07*

*$P < 0.05$ versus FGF2-nTg.

lar density, and decreased cardiomyocyte hypertrophy. Furthermore, FGF2-KO mice demonstrated a complete absence of scar contraction, resulting in increased final infarct size and marked increases in chamber area and infarct expansion. These deficits ultimately impaired cardiac function. Conversely, overexpression of FGF2 was demonstrated to increase fibroblast proliferation and collagen deposition, speed endothelial proliferation, and enhance cardiomyocyte hypertrophy. These changes curbed infarct expansion and preserved LV function.

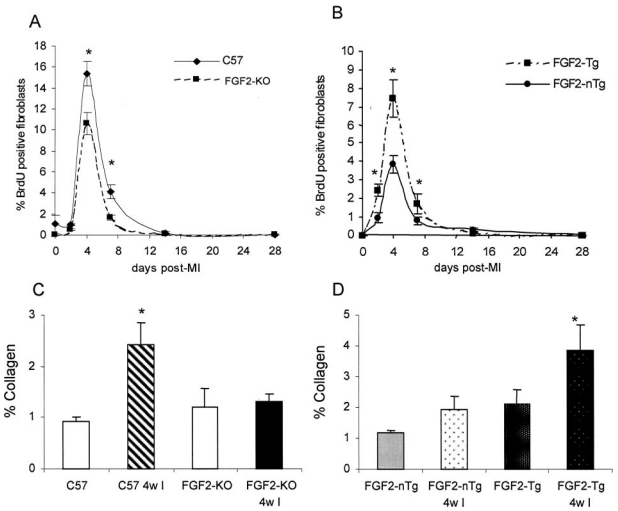

Figure 3. Myofibroblast proliferation and myocardial fibrosis. Proliferating fibroblasts were detected by double labeling with smooth muscle α-actin and BrdU antibodies (see Supplemental Figure 2 at *http://ajp.amjpathol.org* for histological image). Group sizes ranged from 7 to 14 mice per time point (listed in Tables 1 and 2). **A:** Fibroblast proliferation peaked at 4 days after MI in both FGF2-KO and WT mouse hearts; however, there was a 30% reduction in the proliferative response in FGF2-KO hearts (*$P < 0.05$). **B:** Fibroblast proliferation in FGF2-Tg mice was twice that of FGF2-nTg mice at 2 days, 4 days, and 1 week after MI (*$P < 0.05$). **C:** Collagen deposition was measured by picrosirius red staining in the noninfarcted posteroseptal region. Collagen content in FGF2-KO mice did not significantly increase after MI, whereas infarcted WT C57 hearts showed significantly increased interstitial fibrosis (*$P < 0.05$ versus noninfarcted). **D:** Collagen deposition in the FGF2-Tg group after MI was double that of the FGF2-nTg mouse hearts ($P < 0.05$ versus P = ns, respectively). See Supplemental Figure 3 at *http://ajp.amjpathol.org* for histological images.

FGF2 has been studied extensively for its ability to promote angiogenesis in models of chronic ischemia. For example, FGF2 has been reported to increase regional blood flow[15] in dogs and improve ventricular function in pigs with ameroid coronary constrictors.[16–20] FGF2 has also been studied for its role in tissue repair, most commonly in healing of excisional skin wounds.[21,22] Although exogenous administration of FGF family members accelerates wound repair, mice lacking FGF2 or lacking both FGF2 and FGF1 (acidic FGF) showed only a modest slowing of skin wound closure. These findings indicate neither FGF2 nor FGF1 are required for skin wound healing. In contrast, the data presented in the present report demonstrate that FGF2 is a pivotal molecule in controlling myocardial infarct repair. One reason for the difference between skin and heart may lie in the mechanical load under which cardiac repair occurs. As a loose-skinned species, the mouse probably requires less tension from myofibroblasts to contract a skin scar, whereas in the heart, scar contraction occurs under constant wall stress.

A few other studies have also addressed possible roles for FGF2 in myocardial infarct repair, with conflicting results. In rats, systemic administration (intraperitoneal) of FGF2 for 1 week after MI was shown to prevent ventricular dilation and promote hypertrophy of the surviving myocardium,[23] whereas in a subsequent study from the same group, an osmotic pump eluting FGF2 did not affect cell proliferation or ventricular geometry.[24] Some of the discrepancy may result from the bioavailability of protein growth factors, which can be limited by their short half-lives when given systemically. For the current study, we chose to circumvent these issues by genetic deletion or transgenic overexpression.

Role of FGF2 in Cardiac Hypertrophy and Cell Proliferation

We observed a blunted hypertrophic response in the myocytes of the noninfarcted region in FGF2-KO mice, whereas hypertrophy was increased in FGF2-Tg mice.

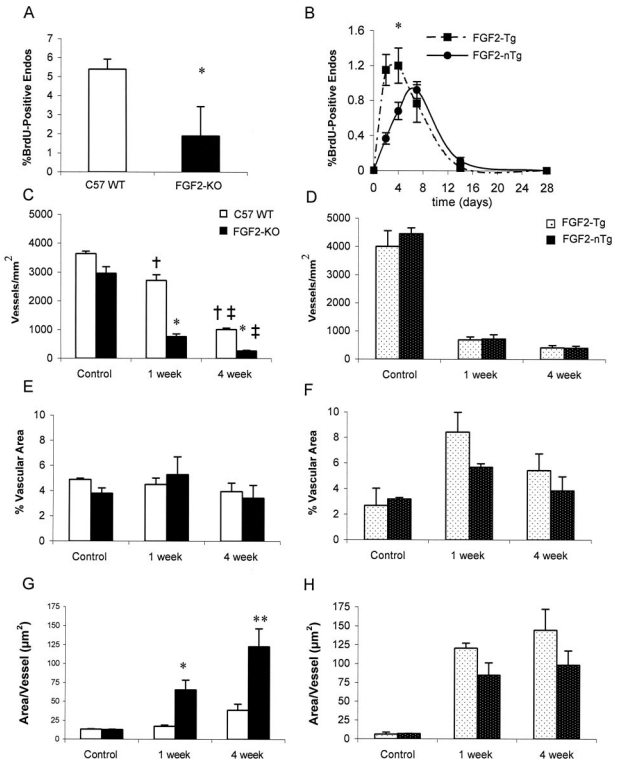

Figure 4. Endothelial cell proliferation and vascular morphometry. Group sizes ranged from 7 to 14 mice per time point, as listed in Tables 1 and 2. **A:** Proliferating endothelial cells were detected by double labeling with CD31 and BrdU antibodies. See Supplemental Figure 2 (available at *http://ajp.amjpathol.org*) for a representative photomicrograph. Endothelial cell proliferation was significantly blunted in FGF2-KO mice, as determined by a cumulative 4-day BrdU infusion ($^{*}P < 0.05$). **B:** Endothelial proliferation in FGF2-Tg mice (determined by pulse labeling 1 hour before sacrifice) peaked earlier than in FGF2-nTg mice ($^{*}P < 0.05$). **C:** In WT hearts, the vascular density declined at 1 week and further declined at 4 weeks after MI ($^{\dagger}P < 0.001$ versus noninfarcted control; $^{\ddagger}P < 0.001$ versus previous time point). Although the initial vascular density was comparable in noninfarcted FGF2-KO hearts, there was a more pronounced decline in density at 1 and 4 weeks after MI ($^{*}P < 0.001$ versus WT C57 at same time point). **D:** There were no differences in vascular density between FGF2-Tg and FGF2-nTg hearts. **E** and **F:** The area occupied by vascular lumen (expressed as a percentage of the total area) did not vary among the time points or between the groups in FGF2-KO or FGF2-Tg studies. **G:** The average area/vessel was significantly increased at 1 ($^{*}P < 0.05$) and 4 weeks ($^{**}P < 0.001$) after MI in the FGF2-KO group compared with WT. **H:** The area/vessel increased comparably in both the FGF2-Tg and FGF2-nTg hearts after infarction.

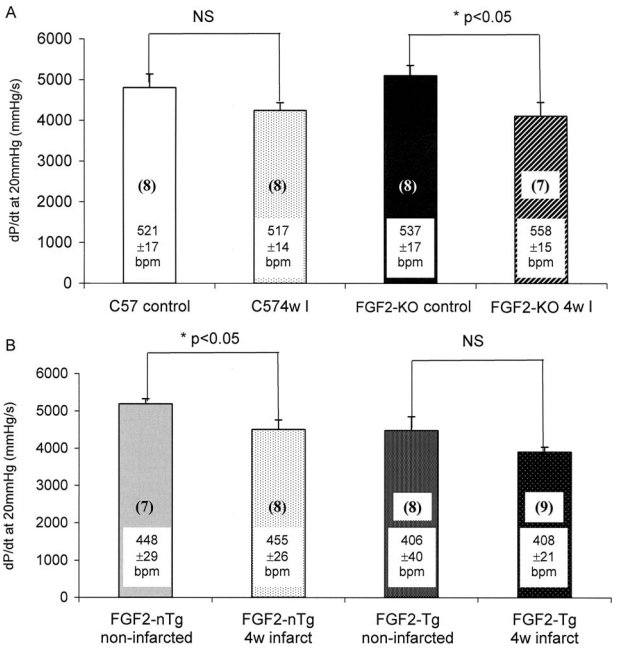

Figure 5. LV function. The derivative of the LV pressure curve at 20 mm Hg was used as the index of contractility (dP/dt). Group sizes are shown inside the bars in parentheses. Heart rates are indicated below the group sizes in beats per minute (bpm). **A:** There was no significant depression of contractile function in WT heart 4 weeks after MI; however, cardiac function was impaired in the FGF2-KO group ($P < 0.05$). **B:** Cardiac function was significantly depressed at 4 weeks after MI compared with control FGF2-nTg hearts ($P < 0.05$); however, there was no significant diminution of contractile function in the FGF2-Tg group.

These findings agree well with previous work in models of hypertension. Transverse aortic coarctation in FGF2-KO mice results in significantly less myocyte hypertrophy compared with WT mice (4 to 24% versus >50%).[6] Similarly, after 4 weeks of renal artery clipping, hypertensive mice lacking FGF2 exhibited more severe chamber dilation, decreased fractional shortening, and did not develop compensatory hypertrophy. Interestingly, *in vitro* experiments implicated cardiac fibroblasts as an important source of FGF2 for hypertrophy and also demonstrated that myocytes from FGF2-KO mice hypertrophied normally in response to exogenous FGF2.[25]

Given the importance of cell proliferation in the formation of cardiac granulation tissue and the demonstrated role for FGF2 in vascular repair,[26,27] we postulated FGF2 would regulate fibroblast proliferation and myocardial fibrosis after infarction. We observed a significant reduction in fibroblast proliferation in FGF2-KO hearts, and we propose that this led to the reduced interstitial collagen deposition and increased infarct expansion in these hearts. Conversely, fibroblast proliferation and resultant fibrosis were greatest in the infarcted FGF2-Tg hearts, and we reason that this contributed to the reduced infarct expansion observed in these hearts.

FGF2 is a well-described angiogenic factor that induces endothelial migration and proliferation. We also observed reduced endothelial proliferation and reduced vascular density in infarcted FGF2-KO hearts. Pintucci and colleagues[28] have reported that migration of FGF2-KO endothelial cells is blunted in response to injury *in vitro*, and this attenuation was mediated by reductions in ERK signaling. Interestingly, FGF2-KO cells are capable of responding to exogenous FGF2, indicating that their downstream signaling pathways remain intact. A surprising finding from our study was that, although the number of vessels was significantly reduced in FGF2-KO infarcts, the vessels that remained were markedly dilated and often devoid of smooth muscle cells (such that total vascular cross-sectional area remained constant). There is growing evidence that endothelial cells recruit mesenchymal cells and direct their differentiation into mural cells through paracrine signaling, and similarly, mural cells promote endothelial cell maturation and survival.[29] We hypothesize that interruption of FGF2 signaling between endothelial and smooth muscle cells resulted in the decreased vessel density and excessively large caliber vessels observed in the FGF2-KO 4-week infarcts.

Interestingly, Bryant and colleagues[30] have reported that anti-FGF-2 antibodies reduced lumen narrowing in a model of coronary artery ligation in mice. Our results are in general agreement with Bryant and colleagues,[30] although the mechanism through which FGF2 controls lumen diameter is unclear. Zhou and colleagues[7] have shown FGF2-KO mice are hypotensive resulting from decreased vascular smooth muscle contractility. After carotid injury, they observed normal cell proliferation, suggesting that FGF2 is more important for regulation of vascular tone. In contrast, our data show reduced proliferation and abnormal vessel remodeling, but we did not observe differences in developed pressure 4 weeks after MI between any of our experimental groups, suggesting that the actions of FGF2 depend on the stimulus.[7]

Role of FGF2 in Infarct Wall Thinning and Ventricular Dilation

According to the law of LaPlace, the regional wall thinning of the infarct and dilation of the LV chamber cause significantly increased wall stress and workload of noninfarcted myocardium.[31] (Indeed, wall stress is proportional to the expansion index.) The markedly worsened infarct expansion observed in FGF2-KO hearts indicates the central role this molecule plays in the infarct repair process. There are probably several components that caused worsened infarct wall thinning and chamber dilation. One clear cause is the failure of FGF2-KO infarcts to undergo scar contraction. Failed scar contraction resulted in a doubling of the infarct size at 4 weeks compared with infarcted WT hearts, which correspondingly increased LV cavity volume. Because myofibroblasts are the cell type responsible for producing the scar and mediating its contraction,[32] the simplest explanation for failed contraction would be reduced infarct fibroblast content because of blunted proliferation. Other mechanisms that could contribute to worsened scar contraction include reduced generation of contractile force per myofibroblast,[32–34] reduced expression of metalloproteinases needed for tissue remodeling,[35] reduced hypertrophy of myocytes in the noninfarcted region, and reduced infarct perfusion because of inhibition of angiogenesis.

Most heart researchers combine gross alterations in ventricular anatomy (wall thinning and chamber dilation) with the histological findings of cardiomyocyte hypertrophy and interstitial fibrosis under the heading of LV remodeling. It is notable that by changing availability of FGF2 one can clearly dissociate these gross and histological findings. FGF2 reduces LV dilation and scar thinning, while simultaneously enhancing cardiomyocyte hypertrophy and interstitial fibrosis during infarct repair, all of which are associated with improved function. Thus, it may be more appropriate to restrict LV remodeling to the gross anatomical changes associated with worsened function and not associate a histological correlate to the term.

Role of FGF2 in Cardiac Function

FGF2 administered before ischemia/reperfusion injury has been reported to activate a signaling pathway that protects acutely against cardiac dysfunction and tissue damage.[36–38] Specifically, this protection has been shown to be mediated by ERK phosphorylation and p38 inhibition in FGF2 transgenic mice;[39] in FGF2-KO mice, protection against cell death and dysfunction was restored by inhibiting JNK.[40] In isolated hearts overexpressing FGF2, myocyte viability was preserved and capillary density increased by 20%.[41] However, in a working heart model of low-flow ischemia, cardioprotection in FGF2 transgenic mouse hearts was shown to be independent of capillary density and coronary flow.[42] Recent studies by Jiang and colleagues[43] have shown that a mutant form of FGF-2 can mediate protection independent of angiogenesis. In our experiments, which used permanent coronary occlusion in a species devoid of collateral blood flow, no acute myocardial protection was predicted or observed. Nevertheless, the structural effects of FGF2 on infarct repair translated into functional differences at 4 weeks. Indeed, infarcted mice lacking FGF2 exhibited poorer LV dP/dt, whereas mice overexpressing FGF2 did not have the decrement in LV dP/dt observed in the appropriate WT controls.

Conclusions

This investigation provides insight into the mechanism of FGF2 action during myocardial infarct repair. We have demonstrated that the lack of FGF2 results in more severe infarct wall thinning and ventricular dilation, blunts myocyte hypertrophy, reduces cell proliferation, fibrosis, and vascular density, and that all these deficits lead to worse cardiac dysfunction 4 weeks after MI. Conversely, overexpression of FGF2 ameliorates these adverse effects and preserves cardiac function. These observations suggest it may be possible to use FGF2 to optimize myocardial infarct repair and enhance cardiac function in patients after MI.

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