Resident Nestin\(^+\) Neural-Like Cells and Fibers Are Detected in Normal and Damaged Rat Myocardium

Viviane El-Helou, Jocelyn Dupuis, Cindy Proulx, Jessica Drapeau, Robert Clement, Hugues Gosselin, Louis Villeneuve, Louis Manganas, Angelino Calderone

Abstract—The present study examined whether nestin\(^+\) neural-like stem cells detected in the scar tissue of rats 1 week after myocardial infarction (MI) were derived from bone marrow and/or were resident cells of the normal myocardium. Irradiated male Wistar rats transplanted with \(\beta\)-actin promoter–driven, green fluorescent protein (GFP)–labeled, unfractonated bone marrow cells were subjected to coronary artery ligation. Three weeks after MI, GFP-labeled bone marrow cells were detected in the infarct region, and a modest number were associated with nestin immunoreactivity. The paucity of GFP\(^+\)/nestin\(^+\) cells in the scar tissue provided the impetus to explore whether neural-like stem cells were derived from cardiac tissue. Nestin mRNA and immunoreactivity were detected in normal rat myocardium, and transcript levels were increased in the damaged heart after MI. In primary-passage, cardiac tissue–derived neural cells, filamentous nestin staining was associated with a diffuse, cytoplasmic glial fibrillary acidic protein signal. Unexpectedly, in viable myocardium, numerous nestin\(^+\)/glial fibrillary acidic protein\(^+\) fiberlike structures of varying length were detected and observed in close proximity to neurofilament-M\(^+\) fibers. The infarct region was likewise innervated, and the preponderance of neurofilament-M\(^+\) fibers appeared to be physically associated with nestin\(^+\) fiberlike structures. These data highlight the novel observation that the normal rat heart contained resident nestin\(^+\)/glial fibrillary acidic protein\(^+\) neural-like stem cells, fiberlike structures, and nestin mRNA levels that were increased in response to myocardial ischemia. Cardiac tissue–derived neural stem cell migration to the infarct region and concomitant nestin\(^+\) fiberlike innervation represent obligatory events of reparative fibrosis in the damaged rat myocardium. (Hypertension. 2005;46:1219-1225.)

Key Words: rats ■ heart ■ myocardial infarction

Fibroblast migration, subsequent transformation to a myofibroblast phenotype, and concomitant nerve fiber innervation represent putative events of reparative fibrosis after cardiac injury.\(^1\)-\(^3\) Subsequent studies performed in the damaged dog and rat myocardium revealed that fibers innervating the infarct region stained positively for neurofilament-M and tyrosine hydroxylase.\(^4\),\(^5\) The prevailing thesis stated that neuronal innervation of the scar occurred, at least in part, by the growth of preexisting fibers originating from the stellate ganglion and facilitated by retrograde transport of the neurotrophin nerve growth factor.\(^5\) However, nerve fiber growth may concomitantly proceed by recruitment of neural stem cells. The intermediate filament protein nestin was identified as a marker of adult neural stem cells located in the central nervous system (CNS), and under the appropriate culture conditions, these cells differentiated to either glial cells or neurons.\(^6\)-\(^8\) Recently, coexpression of glial fibrillary acidic protein in nestin\(^+\) neural stem cells was recognized as an additional phenotype of neural progenitors.\(^9\),\(^10\) In the infarct region of the damaged rat myocardium and in cultured scar-derived cells, nestin immunoreactivity was identified in neural-like cells with numerous processes and/or extensions.\(^5\) The latter data were consistent with the premise that the infarct region contained neural cells that exhibited a stem cell phenotype.\(^5\) Furthermore, nestin and neurofilament-M proteins were coexpressed in a few scar-derived neural cells.\(^5\) Although the latter data did not directly confirm that nestin\(^+\) neural-like cells differentiated to a neuronal phenotype, the observation was consistent with a progenitor role.

Several studies have demonstrated that multipotent stem cell differentiation is not exclusively lineage dependent but in part, is influenced by the environment. Under appropriate culture conditions, skin- and skeletal muscle–derived multipotent stem cells can differentiate to either a mesodermal or a neural stem cell phenotype.\(^11\),\(^12\) A similar paradigm was reported for adult bone marrow–derived stromal cells.\(^13\) With regard to scar-residing, nestin\(^+\) neural-like stem cells, their tissue source remains presently undefined. The current liter-
ature supports the premise that bone marrow cells may have migrated to the scar and subsequently differentiated to a neural stem cell phenotype after exposure to the infarct milieu.12–14 Alternatively, nestin+ neural cells may represent an endogenous stem cell population residing in the normal heart. Consistent with the latter concept, Beltrami et al15 demonstrated that the myocardium contained c-kit+ multipotent cardiac stem cells. Thus, the primary objective of the present study was to elucidate whether nestin+ neural-like stem cells detected in the scar of post–myocardial infarction (MI) rats are derived from bone marrow and/or cardiac tissue.

**Methods**

**MI Model and Isolation of Scar-Derived Cells**

MI was induced in male Sprague-Dawley rats (10 to 12 weeks old; Charles River Laboratories; St. Constant, Quebec, Canada) by ligating the left anterior descending coronary artery, and left ventricular function was measured 1 week after surgery, as previously described.5 The use and care of laboratory rats were conducted according to the Canadian Council for Animal Care and were approved by the Animal Care Committee of the Montreal Heart Institute. Isolation of neural cells and (myo)fibroblasts from the normal left ventricle, noninfarcted left ventricle, and infarct region was performed as previously described.5

**Mouse Follicle-Derived Neural Stem Cells and Brain-Derived Astrocytes**

Mouse follicle-derived neural stem cells were isolated from the entire skin of the dorsum of a P30 C57/BL6 mouse. Cells were removed and digested in trypsin (1 hour, 30°C) and subsequently treated with 2 mg/mL collagenase (2 hours, 37°C). Cells were then grown in poly-2-hydroxyethylmethacrylate–coated dishes in Neurocult basal medium with 10% Proliferation medium ( Stem Cell Technologies) and supplemented with epidermal growth factor and fibroblast growth factor-2 (20 ng/mL added every 2 days; Sigma).16 After neurosphere formation (~14 days), the spheres were collected and plated in uncoated plates containing 7% fetal bovine serum for 1 to 3 days. Plated cells were subsequently subjected to immunofluorescence. Brain-derived astrocytes were provided by Dr. L.-E. Trudeau (Université de Montréal, Département de Pharmacologie).17

**Injection of Green Fluorescent Protein–Labeled Unfractionated Bone Marrow Cells**

Normal male Wistar rats (8 to 10 weeks) were irradiated (7.2 Gy, ~10 minutes; l’Hôpital Maisonneuve-Rosemont, Montreal, Canada), and 24 hours later, β-actin promoter–driven, green fluorescent protein (GFP)–labeled,18 unfractionated adult rat bone marrow cells (5×10^6 cells/mL) were injected into the jugular vein. Flow cytometry of peripheral blood isolated from 4- to 5-week–posttransplanted 1-week post-MI rats was isolated by a modification of the guanidine method described.19 The reverse transcriptase reaction contained 5 ng/μL total RNA (each sample), M-MLV reverse transcriptase (800 U, Invitrogen), RNaseOUT (40 U, Invitrogen), random-hexamer primers (0.04 U, Amberson Biosciences), dNTPs (0.5 mmol/L, MBI Fermentas), and supplied optimal buffers. The reaction protocol consisted of 3 successive incubation steps at (1) 25°C for 10 minutes, (2) 37°C for 50 minutes, and (3) 70°C for 15 minutes.

Real-time polymerase chain reaction (PCR) was performed on 2.5 ng of cDNA template containing the appropriate primers (300 nmol/L) and SYBR Green PCR master mix (Applied BioSystems). Primers for each gene were obtained from distinct exons that spanned an intron by using the Ensembl Genome Browser program (http://www.ensembl.org). The sequence specificity of each primer was verified with the Blast program derived from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The primers used were as follows: for rat atrial natriuretic peptide; forward 5’-AGACGGGACTAGGTCGAAACA-3’ and reverse 5’-ATTGGCTTTATCCTCGGTA-3’; for rat nestin; forward 5’-TGAGCCCACTGATAATTTCA-3’ and reverse 5’-TTCTCTGTCCTCAGGCTTCCA-3’; for rat collagen α1 type 3, forward 5’-ggacgctgtctctcacc-3’ and reverse 5’-AGGTAGTTCAGATCCC-AATTCA-3’; and for rat β-actin, forward 5’-CCCTAAAGGCCCACGCATTGA-3’ and reverse 5’-GAGGCAATACAGGGGCAACACAG-3’. Appropriate negative controls were used for each experiment.

**Immunofluorescence Staining**

The heart was excised, immersed directly in 2-methylbutane (temperature maintained at ~80°C), and stored at ~80°C. Primary- and secondary antibody preparations of scar-derived cells. Data were evaluated by either a Student unpaired t-test or an ANOVA followed by a Newman-Keuls test or an ANOVA followed by a Newman-Keuls post hoc test. A value of P<0.05 considered statistically significant.

**Statistics**

Data are presented as the mean±SEM, and n represents either the number of rats used per experiment or the number of independent preparations of scar-derived cells. Data were evaluated by either a Student unpaired t-test or an ANOVA followed by a Newman-Keuls post hoc test. A value of P<0.05 considered statistically significant.

**Results**

**GFP-Labeled Bone Marrow–Derived Cells Migrated to the Infarct Region**

GFP-labeled, unfractionated bone marrow cells were detected in the scar region of 3-week post-MI irradiated male Wistar rats and appeared interspersed among nestin+ cells (Figure 1). Among the population of grafted GFP+ bone marrow cells identified in the infarct region, 36±12% exhibited nestin immunoreactivity. GFP and nestin coexpression was detected in 22±5% of the total population of scar-residing nestin+ cells.
The paucity of GFP\(^+\)/nestin\(^+\) cells in the infarct region suggested that the majority of scar-residing nestin\(^+\) neural-like stem cells were derived from tissue other than bone marrow. In the normal left ventricle, nestin mRNA was expressed, and nestin\(^+\) immunoreactive cells were detected throughout the myocardium intercalated among well-defined cardiac myocytes (Figure 1). At higher magnification, nestin\(^+\) cells were associated with apparent processes and/or extensions (Figure 1). The latter data were confirmed in vitro, as nestin\(^+\) neural-like cells were present. Phase-contrast photomicrographs of C and D, the latter were confirmed in vitro, as nestin\(^+\) neural-like stem cells with distinct processes and/or extensions were cultured from the normal myocardium (Figure 2). Unexpectedly, organized nestin filaments were detected in a few fibroblasts, and the intensity of the signal was variable (Figure 2). Indeed, the preponderance of fibroblasts did not express nestin. In fibroblasts that exhibited nestin immunoreactivity, the signal was significantly weaker compared with that in nestin\(^+\) neural-like stem cells (Figure 2).
normal myocardium, nestin immunoreactivity was detected in neural-like stem cells cultured from the noninfarcted left ventricle and infarct region (data not shown). Likewise, a weak immunoreactive nestin signal was observed in a modest number of (myo)fibroblasts (data not shown).

Modest numbers of c-kit\textsuperscript{+} cells were observed randomly scattered in the normal rat myocardium and detected intercalated among connexin43\textsuperscript{+} cardiac myocytes (Figure 1). C-kit immunoreactivity was not observed in nestin\textsuperscript{+} cells residing in the normal myocardium (Figure 1). Moreover, the morphological phenotyped of nestin\textsuperscript{+} (processes and/or extensions) and c-kit\textsuperscript{+} (small round shaped) cells were distinct. As previously stated and in contrast to c-kit\textsuperscript{+} cells, nestin staining was detected throughout the normal myocardium (Figure 1). Likewise, in primary-passage scar-derived cells, c-kit immunoreactivity was not detected in nestin\textsuperscript{+} neural-like cells (data not shown).

**Nestin\textsuperscript{+} Neural-Like Stem Cells Expressed Glial Fibrillary Acidic Protein**

In the normal myocardium, glial fibrillary acidic protein immunoreactivity was detected in nestin\textsuperscript{+} cells intercalated among cardiac myocytes (Figure 4). The coexpression of glial fibrillary acidic protein and nestin was reaffirmed in primary-passage neural-like cells derived from the normal myocardium, noninfarcted left ventricle, and infarct region and morphologically resembled putative neural stem cells isolated

<table>
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<tr>
<th>Group</th>
<th>BW, g</th>
<th>LVW/BW, mg/g</th>
<th>RVW/BW, mg/g</th>
<th>SW/BW, mg/g</th>
<th>LVSP, mm Hg</th>
<th>LVEDP, mm Hg</th>
<th>LV + dP/dt, mm Hg/s</th>
<th>LV - dP/dt, mm Hg/s</th>
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<td>Sham (n=5)</td>
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<td>1.41±0.07</td>
<td>0.48±0.02</td>
<td>--</td>
<td>156±9</td>
<td>10±2</td>
<td>7540±435</td>
<td>6270±409</td>
</tr>
<tr>
<td>MI (n=5)</td>
<td>371±9</td>
<td>1.24±0.03</td>
<td>0.50±0.05</td>
<td>0.25±0.01*</td>
<td>133±7</td>
<td>16±1</td>
<td>5920±348*</td>
<td>4120±275*</td>
</tr>
</tbody>
</table>

BW indicates body weight; LVW, left ventricle weight; RVW, right ventricle weight; SW, scar weight; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, rate of contraction; −dP/dt, rate of relaxation. Other abbreviations are as defined in text. Data are presented as mean±SEM.

*P<0.05 vs sham.

**Figure 3.** Gene expression in 1 week post-MI rats. A, Atrial natriuretic peptide (ANP) and collagen-I\textsubscript{α1} mRNA levels were significantly increased in the noninfarcted left ventricle (NILV) and infarct region compared with sham rats. B, Nestin mRNA levels were increased in 1-week post-MI rats. *P<0.05 vs sham, +P<0.05 vs sham and noninfarcted left ventricle. Abbreviations are as defined in text.

**Figure 4.** Glial fibrillary acidic protein expression in nestin\textsuperscript{−} neural-like stem cells. A, Nestin immunoreactivity in the normal left ventricle stained positively for (B) glial fibrillary acidic protein, as reflected by the appearance of a (C) yellow fluorescence. D, Phase-contrast photomicrograph of A, B, and C. E, Nestin\textsuperscript{−} neural-like stem cells cultured from the noninfarcted left ventricle coexpressed unorganized glial fibrillary acidic protein (F). In surrounding fibroblasts (indicated by asterisks), a weak cytoplasmic and nuclear signal of glial fibrillary acidic protein was observed. G, Nestin\textsuperscript{−} intermediate filaments were detected in cultured mouse follicle-derived neural stem cells and were associated with a diffuse cytoplasmic and intense perinuclear glial fibrillary acidic protein signal (H). I, By contrast, glial fibrillary acidic protein intermediate filaments were detected in rat brain-derived astrocytes. Scale bar for A–F and I=20 μm; for G and H=10 μm. Abbreviations are as defined in text.
from the dermis of juvenile mice (eg, numerous processes; Figure 4). However, glial fibrillary acidic protein was not organized in a filamentous pattern but was diffused in the cytoplasm, and in some cells, a nuclear signal was observed (Figure 4). Likewise, in cultured mouse follicle-derived neural stem cells, nestin intermediate filaments were coincident with a diffuse glial fibrillary acidic protein signal in the cytoplasm and an intense perinuclear stain (Figure 4). A nuclear glial fibrillary acidic protein signal was also detected in a modest number of mouse follicle-derived neural stem cells. By contrast, glial fibrillary acidic protein intermediate filaments were evident in rat brain-derived astrocytes, whereas a nuclear signal was absent (Figure 4). Last, a weak, diffuse cytoplasmic and a nuclear glial fibrillary acidic protein signal was observed in fibroblasts derived from the normal and infarcted myocardium (Figure 4).5 Despite the absence of a nonspecific signal in the nucleus after incubation of the cells with the fluorescein isothiocyanate–conjugated secondary antibody alone, the specificity of the nuclear glial fibrillary acidic protein signal remained equivocal.

Nestin*/Glial Fibrillary Acidic Protein+ Fiberlike Structures Were Detected in Normal and Infarcted Rat Hearts, and Scar-Derived Oligodendrocytes Synthesized Nogo-A
Nestin*/glial fibrillary acidic protein+ cells detected in the normal myocardium, peri-infarct region, and infarct region of 1-week post-MI rats were associated with distinct processes and/or extensions of varying length. In the normal and viable myocardium of 1-week post-MI rats, the length of numerous nestin* processes was similar to that of innervating neurofilament-M fibers (Figure 5). Moreover, randomly throughout the myocardium, nestin* fiberlike structures and neurofilament-M* fibers were detected in close proximity. Likewise, nestin* fiberlike structures and neurofilament-M* fibers of similar length were detected innervating the infarct region. However, in contrast to what was observed in viable myocardium, the preponderance of neurofilament-M fibers detected in the infarct region appeared to be physically associated with nestin* fiberlike structures (Figure 5).

In the CNS, the family of peptides designated Nogo (neural outgrowth inhibitors) were characterized as inhibitors of axonal growth and were predominantly synthesized by oligodendrocytes. In neural-like cells cultured from the scar region of 1-week post-MI rats, an oligodendrocyte phenotype was identified, as delineated by the expression of myelin-specific oligodendrocyte protein (MOSP). Moreover, MOSP+ cells and nestin*/glial fibrillary acidic protein+ neural stem cells were morphologically distinct (Figures 2, 4, and 5). Consistent with previous studies, Nogo-A expression was abundant in MOSP+ neural cells (Figure 5). By contrast, a weak, diffuse Nogo-A signal was detected in myofibroblasts. Whether this latter signal represents significant Nogo-A synthesis by myofibroblasts remains equivocal.

Nonneural Nestin Expression in the Infarcted Myocardium
Although the intermediate filament protein nestin was defined as a marker of neural stem cells, expression was also reported in cardiac progenitor cells and endothelial cells during active angiogenesis. In the scar region of 1-week post-MI rats, vessels of variable size were identified, and lumen diameter (long axis) varied between 34 and 193 μm. Nestin immunoreactivity was detected in endothelial cells of vessels with an average lumen diameter of 62±7 μm (range, 34 to 87 μm) (Figure 2). By contrast, in larger vessels (lumen diameter, 150 to 193 μm) with an intact endothelium, nestin staining was either weakly expressed or not detected (data not shown). Nestin immunoreactivity was not observed in vascular smooth muscle cells (Figure 2). Last, nestin staining was not detected in cardiac myocytes of either the normal heart or remote regions of the noninfarcted left ventricle (Figures 1 and 4). However, exclusively within the peri-infarct region, nestin immunoreactivity was observed in some but not all cells that exhibited a striated cardiac myocyte phenotype (Figure 2). Striated cardiac myocytes detected in the peri-infarct region stained positively for α-sarcomeric actin and connexin43 (data not shown).
Discussion
A recent study demonstrated that bone marrow–derived stromal stem cells cultured under the appropriate in vitro conditions differentiated to a neural stem cell phenotype.13 To examine the latter premise, unfractionated, GFP-labeled bone marrow cells were transplanted into irradiated adult male Wistar rats that were subsequently subjected to coronary artery ligation. In 3-week post-MI rats, GFP-labeled bone marrow cells were detected in the infarct region, and nestin immunoreactivity was observed in a modest number of grafted bone marrow cells. Collectively, these data highlight the chemotactic nature of the infarct region and further support the premise that the milieu was capable of promoting nestin expression in a limited population of grafted bone marrow–derived cells. It remains to be confirmed whether bone marrow GFP+/nestin+ cells represent putative neural stem cells. Alternatively, the possibility that cell fusion contributed to the appearance of bone marrow GFP+/nestin+ cells in the infarct region could not be excluded. Regardless of the mechanism, the modest number of bone marrow–derived GFP+/nestin+ cells could not account for the plethora of nestin+ neural-like stem cells residing in the scar region.

The paucity of nestin+/GFP+ cells detected in the infarct region provided the impetus to examine whether scar-residing nestin+ neural-like stem cells were heart-derived cells. Indeed, the latter premise was indirectly confirmed, as GFP expression driven by the nestin second-intron enhancer was identified in the mouse heart.24 Nestin mRNA was detected in the normal rat heart, immunoreactivity was observed intercalated among well-defined cardiac myocytes throughout the myocardium, and nestin+ neural-like stem cells were isolated from the left ventricle. In contrast to multipotent cardiac stem cells,15 c-kit immunoreactivity was not observed in nestin+ cells detected in the normal rat myocardium. In response to coronary artery ligation, nestin mRNA was induced in the noninfarcted left ventricle and further increased in the scar. In the damaged heart of post-MI rats, increased nestin mRNA expression either may reflect induction of the transcript and/or may be secondary to cell proliferation. Thus, the limited numbers of nestin+/GFP+ bone marrow cells detected in the scar region of grafted MI rats support the premise that resident neural stem cells derived from the viable myocardium migrated to and populated the infarct region. Last, modest nestin staining was unexpectedly detected in a few fibroblasts derived from the viable myocardium and infarct region, and intensity was markedly lower, compared with neural-like stem cells.

In the CNS, glial fibrillary acidic protein expression was detected in neural progenitor cells,9,10 and in the present study, immunoreactivity was observed in nestin+ cells detected in the normal and infarcted myocardium. The latter phenotype was likewise confirmed in cultured neural-like stem cells, and their morphology (eg, numerous long processes) resembled those of nestin+ neural stem cells isolated from the skin of juvenile mice.14 However, in both cardiac tissue– and scar-derived, primary-passage, nestin+ neural-like stem cells, glial fibrillary acidic protein immunoreactivity was cytoplasmic and diffuse. Likewise, a diffuse cytoplasmic and perinuclear glial fibrillary acidic protein signal was observed in cultured mouse follicle-derived neural stem cells expressing nestin intermediate filaments.20 In cultured rat brain-derived astrocytes, glial fibrillary acidic protein intermediate filaments were evident. Thus, analogous to CNS- and mouse follicle-derived neural stem cells, glial fibrillary acidic protein was expressed in cardiac tissue- and scar-derived primary-passage nestin+ neural-like stem cells. The diffuse cytoplasmic pattern of glial fibrillary acidic protein expression may represent an underlying feature distinguishing neural stem cells and mature astrocytes. Last, tissue damage and subsequent scar formation in the CNS were associated with the recruitment of glial fibrillary acidic protein–expressing astrocytes that reexpressed both vimentin and nestin and were redefined as reactive astrocytes.7,25 In our previous study, scar-derived neural cells that coexpressed nestin and glial fibrillary acidic protein were considered reactive astrocytes.5 However, based on the findings of the present study, the coexpression of nestin and unorganized glial fibrillary acidic protein may instead reflect a neural-like stem cell phenotype.

Coincident with individual neural-like stem cells, nestin+/glial fibrillary acidic protein+ fiberlike structures of varying length were detected innervating the normal myocardium, noninfarcted left ventricle, and infarct region. Likewise, in the dentate gyrus of the adult mouse brain, nestin+/glial fibrillary acidic protein+ processes were detected.10 Two populations of nestin+ cells were identified and defined as type I and type II. The type I cells were glial fibrillary acidic protein+ with longer processes and were considered early neural progenitors.10 Moreover, randomly throughout the normal and viable myocardium of 1-week post-MI rats, nestin+/glial fibrillary acidic protein+ fiberlike structures and neurofilament-M+ fibers were detected in close proximity. Likewise, nestin+/glial fibrillary acidic protein+ fiberlike structures and neurofilament-M+ fibers of similar length were also observed innervating the infarct region. However, the preponderance of neurofilament-M fibers detected in the infarct region appeared to be physically associated with nestin+/glial fibrillary acidic protein+ fiberlike structures. Presently, it remains equivocal as to whether the latter physical association identified in the infarct region represents either an interaction between 2 distinct fibers or coexpression of nestin/glial fibrillary acidic protein and neurofilament-M filaments within the same fiber.

Scar innervation may have occurred by growth of preexisting nestin+/glial fibrillary acidic protein+ and/or neurofilament-M+ fibers residing in the viable myocardium of the infarcted rat heart. At least with regard to neurofilament-M fiber growth, the latter process was suggested to occur by locally synthesized neurotrophins.4,5 However, fiber innervation of the scar may be partially antagonized by the local production of axonal growth inhibitors. In the CNS, Nogo-A synthesis by oligodendrocytes attenuated axonal growth and/or sprouting.21 In primary-passage, scar-derived cells, nestin+/glial fibrillary acidic protein+ and MOSP+ cells were morphologically distinct, and Nogo-A expression was abundantly expressed in the latter cells. Thus, the extent of nestin+/glial fibrillary acidic protein+ and/or neurofilament-M fiber innervation of the infarct region may reflect a dynamic interaction between locally synthesized neurotrophins and axonal growth inhibitors.
In addition to neural stem cells, nestin expression was also detected in pancreatic stem cells, endothelial cells during active angiogenesis, and myocardium-derived c-kit progenitor cells. Consistent with an angiogenic response during reparative fibrosis, numerous vessels of various size were identified in the scar region of 1-week post-MI rats. Nestin staining was detected in endothelial cells of vessels with an average lumen diameter of 62 μm, whereas a signal was absent in the surrounding vasculature. These data further reaffirm the premise that nestin expression may highlight ongoing angiogenesis, and elevated mRNA levels detected in the infarct region did not exclusively reflect neural stem cell recruitment and/or proliferation. Last, scattered in the peri-infarct region, some but not all striated cardiac myocytes stained positively for nestin. By contrast, nestin immunoreactivity was not detected in cardiac myocytes residing in either the normal left ventricle or remote regions of the noninfarcted left ventricle. It is tempting to speculate that the modest number of striated nestin cardiac myocytes detected exclusively in the peri-infarct region was derived from c-kit multipotent cardiac stem cells.

The present study has provided the novel observation of resident neural-like stem cells in the normal rat heart identified by the coexpression of nestin and glial fibrillary acidic protein. Nestin/glial fibrillary acidic protein fiberlike structures were detected in the normal myocardium, the noninfarcted left ventricle, and the infarct region. The morphology and phenotype of cardiac tissue–derived nestin/glial fibrillary acidic protein fiberlike structures resembled early neural progenitors identified in the dentate gyrus of the adult mouse brain tentatively implicated in neurogenesis. Whether a similar paradigm is prevalent in the normal and infarcted myocardium remains to be elucidated. Thus, cardiac tissue–derived neural stem cell migration to the infarct region and concomitant nestin/glial fibrillary acidic protein fiberlike innervation represent obligatory events of reparative fibrosis in the damaged rat myocardium.

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References
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