

## Genetic Manipulation of Periostin Expression in the Heart Does Not Affect Myocyte Content, Cell Cycle Activity, or Cardiac Repair

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**Abstract**—Following a pathological insult, the adult mammalian heart undergoes hypertrophic growth and remodeling of the extracellular matrix. Although a small subpopulation of cardiomyocytes can reenter the cell cycle following cardiac injury, the myocardium is largely thought to be incapable of significant regeneration. Periostin, an extracellular matrix protein, has recently been proposed to induce reentry of differentiated cardiomyocytes back into the cell cycle and promote meaningful repair following myocardial infarction. Here, we show that although periostin is induced in the heart following injury, it does not stimulate DNA synthesis, mitosis, or cytokinesis of cardiomyocytes *in vitro* or *in vivo*. Mice lacking the gene encoding periostin and mice with inducible overexpression of full-length periostin were analyzed at baseline and after myocardial infarction. There was no difference in heart size or a change in cardiomyocyte number in either periostin transgenic or gene-targeted mice at baseline. Quantification of proliferating myocytes in the periinfarct area showed no difference between periostin-overexpressing and -null mice compared with strain-matched controls. In support of these observations, neither overexpression of periostin in cell culture, via an adenoviral vector, nor stimulation with recombinant protein induced DNA synthesis, mitosis, or cytokinesis. Periostin is a regulator of cardiac remodeling and hypertrophy and may be a reasonable pharmacological target to mitigate heart failure, but manipulation of this protein appears to have no obvious effect on myocardial regeneration. (*Circ Res.* 2009;104:e1-e7.)

**Key Words:** fibroblast ■ remodeling ■ periostin ■ cardiomyocyte proliferation ■ myocardial infarction

Myocardial infarction (MI) leads to an irreversible loss of cardiomyocytes, with expansion and remodeling of the extracellular matrix (ECM) and scar formation. This “fibrous scar” lacks contractile capacity and results in adverse changes to chamber geometry, global systolic dysfunction, and poor ventricular compliance. If adult cardiomyocytes could proliferate and repopulate the injured area, cardiac function may be rescued, although the ability of cardiomyocytes to proliferate in a physiological meaningful manner remains an area of ongoing controversy.<sup>1–3</sup> Recent reports have proposed that a population of postmitotic cardiomyocytes, if exposed to the proper environment, may be capable of proliferation.<sup>3–6</sup>

Although a targeted therapy to drive cardiomyocyte proliferation has not been reported, it may be possible given that detectable, albeit extremely small, increases in cardiomyocyte cell cycle reentry can occur after myocardial injury.<sup>3,5,7,8</sup> The number of cardiomyocytes that enter the cell cycle at baseline, evident by markers for DNA synthesis, occurs at a very low frequency of  $\approx 0.008\%$ .<sup>8</sup> The trigger that drives this small percentage of cells to proliferate has not yet been determined. A recent publication has proposed that the ECM protein periostin leads to the initiation of DNA synthesis,

mitosis, and ultimately cytokinesis.<sup>9</sup> These results have sparked controversy among those interested in the function of periostin because most other reports have only observed an effect on the ECM and collagen fibrillogenesis.<sup>10,11</sup>

Periostin is a 90-kDa secreted protein that contains 4 fasciclin domains<sup>12,13</sup> and is similar to the insect protein fasciclin I, which is involved in neuronal cell–cell adhesion.<sup>12</sup> Periostin is detectable in the developing heart but is not present in the adult ventricular myocardium at baseline.<sup>14–19</sup> It is reexpressed exclusively by fibroblasts or cells that adopt a fibroblast-like phenotype following an injurious event such as MI or pressure overload stimulation.<sup>20–23</sup> Using transgenic and gene-deleted mice, periostin was shown to affect cardiac hypertrophy and ventricular remodeling.<sup>15</sup> Periostin-null (*Pn*<sup>-/-</sup>) mice are prone to rupture following MI, but if they survive the first 10 days of this insult, their cardiac morbidity is improved compared with wild-type mice.<sup>15</sup> These data support the hypothesis that periostin orchestrates cellular remodeling and collagen deposition in the heart. Periostin also functions in a similar fashion when secreted by neoplastic cells.<sup>24–26</sup> An increased concentration of periostin correlates with enhanced cellular migration and adhesion and is

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proportional to the cancer cells ability to invade and metastasize.<sup>24–28</sup>

Although the exact role of periostin has not been defined, the contention that it mediates physiological meaningful myocyte proliferation in the heart following MI is currently a matter of great interest considering the therapeutic ramifications. Here, we used transgenic mice to determine whether the presence of periostin leads to cardiomyocyte proliferation or whether its absence in gene-targeted mice antithetically reduces this index. We also examined a model of cellular proliferation in cultured neonatal cardiomyocytes, which have a finite capacity for proliferation. In both models, we determined that periostin had no effect on any aspect of cell cycle reentry in cardiomyocytes.

## Materials and Methods

### Animals

All mouse protocols were approved by the Institutional Animal Care and Use Committee of Cincinnati Children's Hospital Medical Center.  $Pn^{-/-}$  and transgenic overexpressors ( $Pn^{tTA}$ ) have been described previously.<sup>15</sup>

### Western Blot Analysis

Western blot analysis confirmed overexpression of periostin protein in  $Pn^{tTA}$  mice and its absence in  $Pn^{-/-}$  mice. A rabbit polyclonal antibody to periostin was used.<sup>24</sup> Western blotting procedures were described previously.<sup>29</sup>

### MI and 5-Bromodeoxyuridine Labeling

MI was performed by permanent ligation of the left coronary artery as previously described.<sup>30</sup> Because of the small size of the  $Pn^{-/-}$  mice, body weight-matched 10- to 12-week-old  $Pn^{-/-}$  mice (23.4±0.6g) were used for MI, whereas 8-week-old C57BL/6 mice (24.1±0.5g) were used as controls. Because the growth of the  $Pn^{tTA}$  mice is not affected, 8- to 10-week-old  $Pn^{tTA}$  mice and FVB littermates were used for MI. Seven days after MI, mice received IP injections with 5-bromodeoxyuridine (BrdUrd) labeling reagent (1 mL/100g) (Zymed). Four hours after injection, organs were harvested, fixed in buffered 10% formalin, embedded in paraffin, and cut into 6- $\mu$ m sections. Duodenum was harvested and fixed as a positive BrdUrd-labeling control.

### Immunohistochemistry

Sections, 6  $\mu$ m thick, were deparaffinized and incubated with primary antibody to BrdUrd (Sigma). Slides were stained with wheat germ agglutinin (WGA)–fluorescein-4-isothiocyanate (FITC) (Sigma) to distinguish the sarcolemma membrane. Nuclei were stained with TO-PRO-3 (Molecular Probes). Fluorescent secondary antibody was used (Molecular Probes), and slides were examined with confocal microscopy. To determine the number of cells positive for phosphorylated histone H3 (Cell Signaling), immunohistochemistry was performed following antigen retrieval with sodium citrate. These slides were also stained with WGA-FITC and TO-PRO-3. The periinfarct areas of 4 animals, >100 000 cells, were counted per cohort for both BrdUrd and phosphorylated histone H3. Data were presented as percentage of positive cardiomyocyte nuclei versus total cardiomyocyte nuclei.

### Histology and Cell Surface Area Measurements

Hearts were harvested and fixed in 10% buffered formalin, embedded in paraffin, and cut into 6- $\mu$ m sections. Sections were stained for fibrosis with Masson's trichrome or with WGA for the sarcolemma membrane. Measurement of myocyte cross-sectional area was counted from both the apex and the center region of the left ventricle, in a minimum of 3 separate hearts from each cohort; >2000

cardiomyocytes were measured. Cross-sectional area was calculated using ImageJ software.

## Recombinant Adenovirus and In Vitro Proliferation Experiments

A periostin recombinant adenovirus, AdPn, was generated using the full-length mouse periostin, subcloned into the shuttle vector for the Adeasy system (Invitrogen). All in vitro experiments were performed in primary neonatal rat cardiomyocytes and fibroblasts, which were isolated from 1- to 2-day-old neonates and differentially plated as described previously.<sup>31</sup> The cardiomyocytes were initially plated in 2% FBS in M199 media on gelatin coated chamber slides and infected/stimulated on day 2, after which the serum was reduced for experimentation of proliferation rates. For adenoviral infection, cardiomyocytes and fibroblasts were incubated for 2 hours with AdPn and AdGFP. The positive control cells were grown in media supplemented with 15% FBS; all other conditions were performed in 0.2% FBS media. Similar methods and conditions were used for the neonatal cardiac fibroblasts. To determine the effect of periostin, cells were infected with AdPn or stimulated daily with recombinant human periostin, a C-terminal truncated version of the full-length protein (BioVendor, Czech Republic). The source of recombinant periostin is the same as used by Kühn et al.<sup>9</sup> AdGFP and vehicle controls were also used under identical conditions. Infection efficiency was examined with AdGFP infection. Forty-eight hours following infection, >95% of the cells were GFP positive. A similar dosage of AdPn infection was used to achieve >95% infectivity. To assess proliferation, cells were labeled with BrdUrd for the final 24 hours of experimentation. To detect DNA synthesis, immunohistochemistry for BrdUrd was performed, along with cell type-specific markers for cardiomyocytes ( $\alpha$ -actinin [Sigma] and troponin [Cell Signaling]); fibroblasts were detected with vimentin (Sigma) antibody. Further immunohistochemistry was performed with antibodies to phosphorylated histone H3 and aurora B kinase (Abcam) to detect mitosis and cytokinesis, respectively. Fluorophore-conjugated secondary antibodies were provided by Invitrogen. Proliferating cells in culture were quantified (N>1000), and data are presented as percentages of positive nuclei versus total nuclei. Each experiment was repeated in quadruplicate.

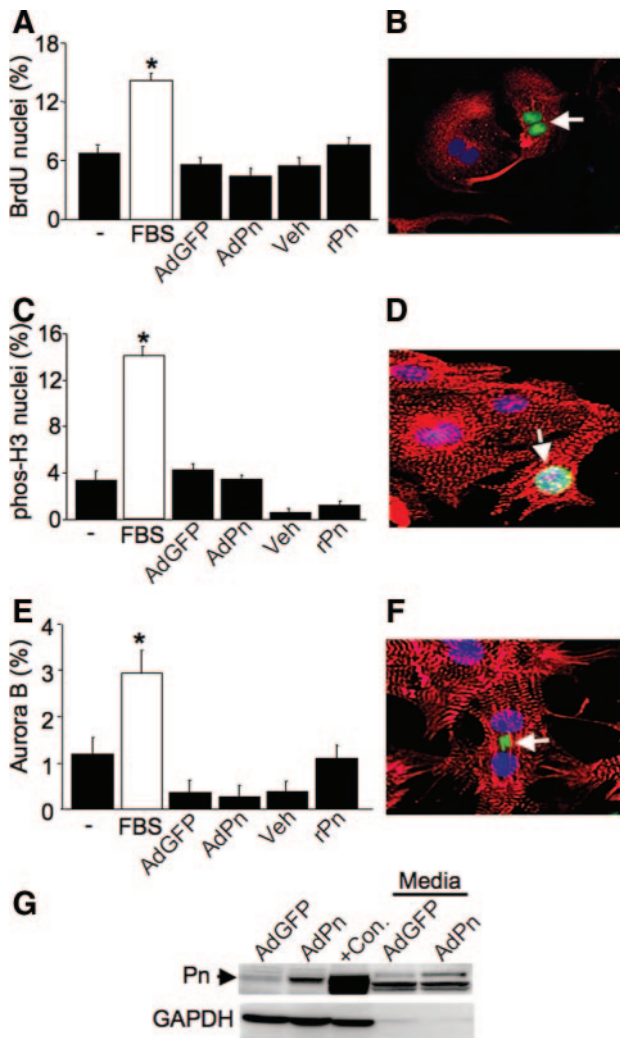
### Statistics

All data are expressed as means±SEM. Statistical significance was determined with a paired Student *t* test between 2 groups or 1-way ANOVA where appropriate. Probability values of <0.05 were considered statistically significant.

## Results

### Periostin Does Not Induce Neonatal Cardiomyocyte or Fibroblast Proliferation In Vitro

A previous report concluded that fibroblasts produce periostin to promote neighboring cardiomyocytes to reenter the cell cycle in the heart.<sup>9</sup> To examine this potential phenomenon in greater detail, in vitro experiments were first performed with isolated neonatal cardiomyocytes because they possess some proliferative capacity at baseline, suggesting they would be permissive to cell cycle reentry. Cardiomyocytes were pulse-labeled with BrdUrd to detect DNA synthesis. As a positive control, 15% FBS was used, which induced a 2.1-fold increase in BrdUrd-positive cardiomyocyte nuclei after 48 hours compared with 0.2% FBS cultured myocytes. However, overexpression of periostin by infection with a recombinant adenovirus, AdPn, did not increase DNA synthesis rates compared with AdGFP infection (Figure 1A and 1B). AdPn treatment for 3 days also did not increase DNA synthesis (data not shown). Treatment with recombinant periostin also



**Figure 1.** Neonatal cardiomyocyte proliferation is not altered by periostin. A, Immunocytochemistry detection of BrdUrd incorporation in neonatal cardiomyocytes vs total cardiomyocyte nuclei. Positive control was 15% FBS and the negative control (-) was no stimulation. B, Image of representative BrdUrd-positive cardiomyocyte nuclei. Red is troponin (myocyte), blue is nuclei, and green is BrdUrd (arrow). C and D, Immunocytochemical quantification and representative image of cardiomyocyte nuclei positive for phosphorylated histone H3. Red is troponin (myocyte), blue is nuclei, and green is phosphorylated histone H3 (arrow). E and F, Immunocytochemical quantification and representative image of cardiomyocyte nuclei positive for aurora B kinase staining compared with total cardiomyocyte nuclei. Red is troponin (myocyte), blue is nuclei, and green is aurora B kinase (arrow). G, Western blot for periostin protein from AdPn-infected cardiomyocytes vs AdGFP infection or concentrated conditioned media 48 hours after AdPn or AdGFP infection. The positive control (+Con) is protein extract from 2-day-old neonatal feet. \* $P < 0.05$  positive vs negative control. rPn indicates recombinant periostin; Pn, periostin; veh, vehicle.

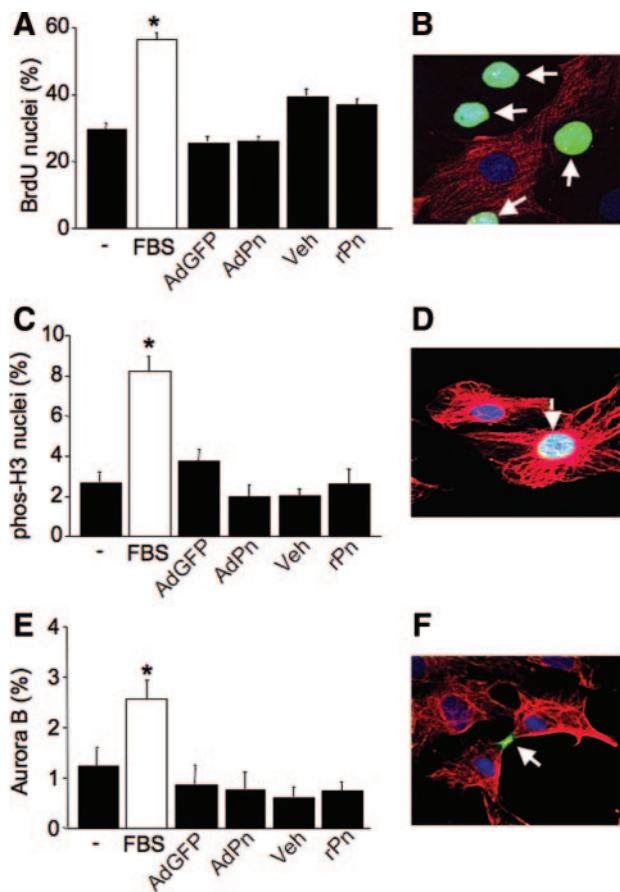
did not induce a significant change in DNA synthesis (Figure 1A and 1B). Because neonatal cardiomyocyte preparations are rarely free of fibroblasts, myocytes were identified by expression of  $\alpha$ -actinin and/or troponin. We further characterized cardiomyocyte proliferation by using specific immunohistochemical markers of mitosis and cytokinesis. The percentage of nuclei positive for phosphorylated histone H3, a marker for mitosis, was not significantly different between

the AdPn-infected cells or with recombinant periostin compared with their respective controls (Figure 1C and 1D). The percentage of positive cells treated with recombinant periostin and vehicle was lower than with the negative control, possibly attributable to the pH of the buffer required to reconstitute the protein. Cytokinesis, the final event of proliferation, was detected by aurora B kinase staining. Once again, neither infection with AdPn nor treatment with exogenous periostin significantly increased the percentage of cardiomyocytes undergoing cytokinesis, although serum stimulation was readily effective (Figure 1E and 1F). To rule out posttranslational processing as a requirement for bioactivity, we also collected conditioned media of myocytes and fibroblasts infected with AdPn to incubate with freshly prepared neonatal myocytes for 48 hours, but, once again, no effect on DNA synthesis or other indexes of proliferation was observed (data not shown). As a control, AdPn infection of cardiomyocytes and conditioned media from AdPn-infected cardiomyocytes both showed expression of full-length periostin by Western blotting (Figure 1G).

To further delineate the role of periostin in inducing cell cycle reentry, we repeated the above experiments with neonatal cardiac fibroblasts. AdPn and recombinant periostin did not stimulate DNA synthesis in fibroblasts, whereas the positive control of 15% FBS was efficacious (Figure 2A and 2B). Phosphorylated histone H3 staining revealed a 3-fold increase in the percentage of cells undergoing mitosis in the 15% FBS group, whereas AdPn and recombinant periostin did not significantly alter this index of cellular proliferation (Figure 2C and 2D). Furthermore, AdPn and recombinant periostin had no effect on cellular mitosis, as detected by aurora B kinase immunohistochemistry (Figure 2E and 2F). In the myocardium, fibroblasts and cardiomyocytes coexist and are immersed in the same milieu of growth factors and proteins; hence, additional coculture experiments were performed. Neonatal rat cardiac fibroblasts were infected with AdPn and were subsequently cultured with neonatal rat cardiomyocytes. Markers of cell cycle reentry (BrdUrd, phosphorylated histone H3, and aurora B kinase) were examined and revealed no significant change in the quantity of cells proliferating (data not shown). Collectively, these data indicate that periostin cannot induce cell cycle reentry in 2 different cell types that are normally permissive to some level of proliferation.

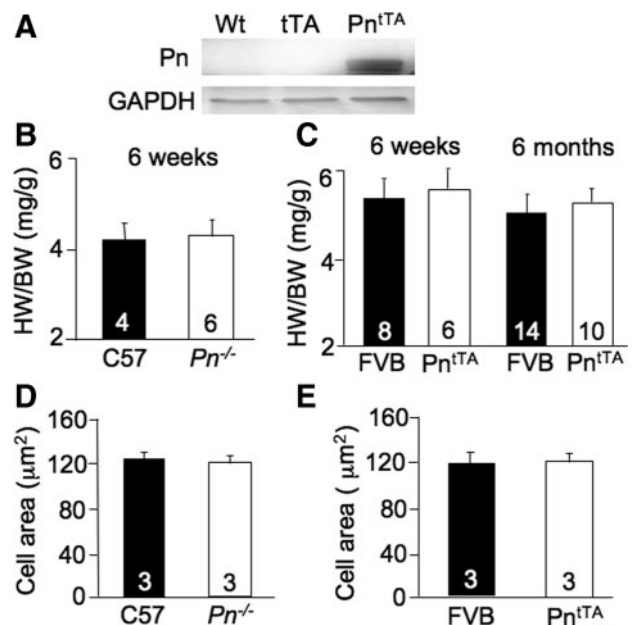
### Periostin-Null and Overexpressing Mice Do Not Have Altered Myocyte Numbers in the Heart at Baseline

We also reasoned that if periostin directly regulated myocyte proliferation, then it would most likely do so throughout development when these cells are readily dividing. Indeed, a large number of genetically modified mouse models with altered cell cycle regulatory proteins have been created that display significant changes in total myocyte cell numbers attributable to a developmental effect of these proteins.<sup>32–35</sup> However, baseline characterization of the  $Pn^{-/-}$  and periostin-overexpressing transgenic ( $Pn^{TA}$ ) mice at 6 weeks of age showed no difference in total cell number in the heart. At 6 weeks of age, periostin is not detectable in the heart of



**Figure 2.** Neonatal fibroblast proliferation is not altered by periostin. A, Immunocytochemistry detection of BrdUrd incorporation in primary fibroblasts vs total fibroblast nuclei. The positive control was 15% FBS and the negative control (-) was no stimulation. B, Image of a representative BrdUrd-positive fibroblast nuclei. Red is troponin to show contaminating myocytes, blue is nuclei, and green is BrdUrd (arrows). C and D, Immunocytochemical quantification and representative image of fibroblast nuclei positive for phosphorylated histone H3. Red is vimentin (fibroblast), blue is nuclei, and green is phosphorylated histone H3 (arrow). E and F, Immunocytochemical quantification and representative image of fibroblast nuclei positive for aurora B kinase staining compared with total fibroblast nuclei. Red is vimentin (fibroblast), blue is nuclei, and green is aurora B kinase (arrow). \* $P < 0.05$  positive vs negative control. rPn indicates recombinant periostin; Pn, periostin; veh, vehicle.

the wild-type or  $Pn^{-/-}$  mice (data not shown) but is abundantly present in the hearts of the  $Pn^{tTA}$  mice (Figure 3A). Importantly, the heart weight to body weight ratio was not altered in the  $Pn^{-/-}$  animals ( $4.3 \pm 0.5$  mg/g) versus age-matched controls ( $4.2 \pm 0.4$  mg/g) (Figure 3B) or  $Pn^{tTA}$  transgenic animals ( $5.1 \pm 0.2$  mg/g) versus appropriate controls ( $4.8 \pm 0.3$  mg/g).  $Pn^{tTA}$  mice at 6 months of age also showed no significant difference in heart size normalized to body weight ( $5.4 \pm 0.2$  mg/g) when compared with controls ( $5.3 \pm 0.3$  mg/g; Figure 3C). To calculate total myocyte number in the heart, the cross-sectional area of myocytes measured from ventricular histological sections was analyzed at 6 weeks of age and determined to be similar between the  $Pn^{-/-}$ ,  $Pn^{tTA}$ , and their controls (Figure 3D and 3E). Cell number and myocyte fraction was assessed by immunohistochemistry, and, once again, no differences were noted be-

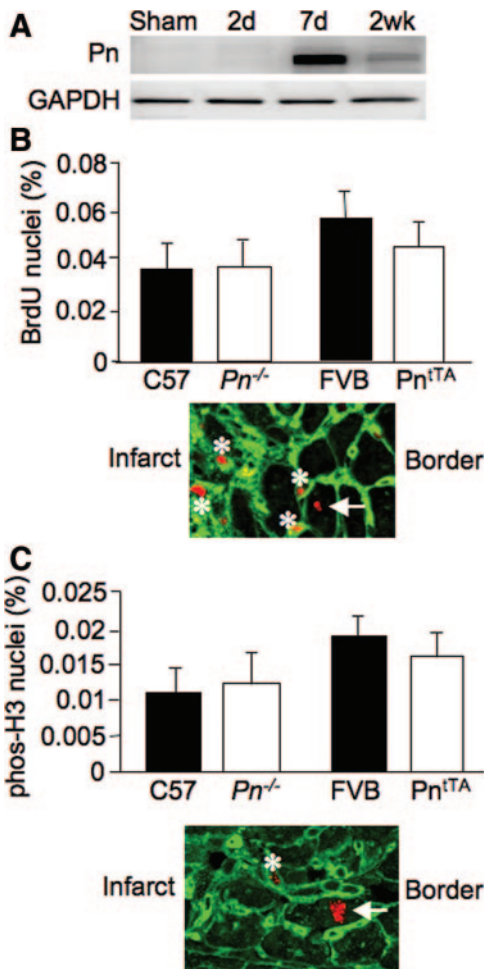


**Figure 3.** Baseline myocyte content is not different in the hearts of  $Pn^{-/-}$  and  $Pn$  transgenic mice. A, Western blot for periostin (Pn) from hearts of 6-week-old FVB, tTA, and  $Pn^{tTA}$  mice. GAPDH is a loading control. B, Assessment of heart weight (HW) normalized to body weight (BW) in  $Pn^{-/-}$  vs wild-type (C57BL/6) mice at 6 weeks of age. C, HW/BW in  $Pn^{tTA}$  and wild type (FVB) at 6 weeks and 6 months of age. D and E, Assessment of myocyte cross-sectional cell areas from left ventricular histological sections of the indicated genotypes ( $n=3$  hearts each, with a minimum of 2000 cells counted).

tween any of the groups (data not shown). Additionally, the degree of fibrosis was not significantly different in the hearts of 6-week-old  $Pn^{tTA}$  mice and age-matched controls, as assessed by Masson's trichrome staining of histological sections (data not shown).

### Periostin Does Not Induce Cardiomyocyte Proliferation In Vivo Following MI

Following myocardial injury, there is abundant reexpression and accumulation of periostin in the interstitial space within and surrounding the injured area.<sup>15</sup> Periostin protein is detected as early as 4 days after MI and peaks at 7 days and remains detectable for up to 8 weeks.<sup>15,16</sup> To examine whether periostin expression and accumulation in the ECM stimulates cardiomyocyte cell cycle reentry in vivo, MI injury was induced in both the  $Pn^{-/-}$  and  $Pn^{tTA}$  transgenic mice and their appropriate wild-type controls. There was no difference in infarct size between the  $Pn^{-/-}$  mice, the  $Pn^{tTA}$  mice, and their respective controls in agreement with our previous results (data not shown).<sup>15</sup> Histological analysis was performed 7 days after MI, when periostin expression is maximal (Figure 4A). Four hours before harvesting the heart, animals underwent an intraperitoneal injection of BrdUrd to label cardiomyocytes that were undergoing DNA synthesis. On examination of the periinfarct area of control mice (C57BL/6), consistent with published data, there was a very small number (0.035%) of BrdUrd-positive cardiomyocyte nuclei.  $Pn^{-/-}$  and  $Pn^{tTA}$  transgenic mice did not show a significant change in cardiomyocyte DNA synthesis (Figure 4B). A representa-



**Figure 4.** Assessment of cardiomyocyte proliferation 7 days after MI in *Pn*<sup>-/-</sup> and *Pn*<sup>tTA</sup> transgenic mice. A, Western blot analysis of periostin in the heart following MI. GAPDH is a loading control. B, Percentage of BrdUrd-positive cardiomyocyte nuclei when compared with total cardiomyocyte nuclei in the periinfarct area of *Pn*<sup>-/-</sup> mice, *Pn*<sup>tTA</sup> mice, and their respective controls. Below the graph a representative immunohistological image is shown with labeled nonmyocytes (asterisk) and a labeled myocyte (arrow). C, Percentage of phosphorylated histone H3-positive cardiomyocytes compared with total cardiomyocytes in the periinfarct area of *Pn*<sup>-/-</sup> mice, *Pn*<sup>tTA</sup> mice, and their respective controls. Below the graph, a representative immunohistological image is shown with a labeled nonmyocyte (asterisk) and a labeled myocyte (arrow). Experiments were performed on a minimum of 4 hearts per cohort. Greater than 100 000 cells were counted per experimental group. None of the groups was significantly different.

tive immunohistochemical image from the infarct border zone is shown with cells outlined with WGA-FITC and BrdUrd labeling, which revealed nonmyocytes (asterisk) and a single labeled cardiomyocyte (arrow) as undergoing DNA synthesis (Figure 4B). To further support these results, immunohistochemical analysis was performed to detect phosphorylated histone H3 staining as evidence of mitosis. Only 0.012% of myocyte nuclei were positive for phosphorylated histone H3 in the periinfarct area. There was no significant increase in the percentage of positive nuclei in either *Pn*<sup>-/-</sup> or *Pn*<sup>tTA</sup> mice (Figure 4C). A representative immunohistochemical image from the infarct border zone is shown with cells outlined with WGA-FITC and phosphorylated histone

H3 labeling, which revealed a labeled nonmyocyte (asterisk) and cardiomyocyte (arrow) (Figure 4C). A minimum of 4 mice was analyzed in each group. These results indicate that overexpression of periostin or its loss does not appreciably affect myocyte cell cycle activity in the periinfarct region.

## Discussion

Although adult cardiac myocytes are not thought to proliferate in vivo given their highly differentiated state, a finite population are proposed to reenter the cell cycle following experimental MI.<sup>8</sup> Such myocytes are typically located in the periinfarct area and are presumably responding to external stimuli, such as growth factors or changes in ECM composition. Our rates of endogenous cardiomyocyte cell cycle activity in the periinfarct region of hearts after MI were comparable to previous accounts in the literature (0.005% - 0.04%),<sup>5,8,36</sup> which given the extremely low value is unlikely to contribute to cardiac regeneration in a meaningful manner. We also used an in vitro model in which neonatal cardiomyocytes were isolated from 1 to 2 day old rat pups. This model was selected because neonatal myocytes inherently possess some degree of cell cycle activity, suggesting they are physiologically primed to respond to proliferative signals if they occur. Indeed, serum stimulation promoted a greater than 2-fold activation of cell cycle activity in these myocytes. However, in neither context did periostin alter myocyte cell cycle activity or proliferation.

As a secreted ECM protein that is associated with areas of fibrosis, remodeling, and inflammation, periostin can directly interact with other ECM proteins such as fibronectin, tenascin-C, collagen I, collagen V, and heparin.<sup>10,11,23</sup> Periostin serves as a ligand for select integrins, such as  $\alpha_5\beta_3$ ,  $\alpha_v\beta_5$ , and  $\alpha_4\beta_6$ , where it can affect the ability of cells (fibroblasts or cancer cells) to migrate and/or undergo an epithelial-mesenchymal transition in select tissues.<sup>24,25</sup> MI or pressure overload stimulation to the adult heart induces abundant reexpression of periostin from resident fibroblasts located between myocytes within the heart parenchyma proper, where, before such stimulation, the only observed expression of periostin in the heart is within the collagen-rich environment of the valves.<sup>14-19</sup> Periostin expression is also induced at sites of vascular injury,<sup>37,38</sup> in the lung after injury/fibrosis,<sup>23,39</sup> in and around tumors,<sup>39</sup> and at wound sites.<sup>40</sup> Although not unequivocal, these various studies suggest a common biological response whereby periostin becomes reexpressed at injury or inflammatory sites within the adult organism, often associated with a need for ECM remodeling and cellular migration, not unlike many developmental processes associated with periostin expression.

The function of periostin on reexpression in the injured myocardium is currently an area of controversy given disparate accounts in the literature. We originally reported that *Pn*<sup>-/-</sup> mice had an altered fibrotic response and were unable to form proper scars after MI injury, leading to greater rates of LV rupture and death.<sup>15</sup> This result was independently confirmed the following year in separately generated *Pn*-null mice.<sup>16</sup> Conversely, we generated transgenic mice overexpressing full-length periostin in the heart, which were protected from wall rupture following MI injury.<sup>15</sup> Abundant

periostin overexpression in the heart did not induce fibrosis, although it did eventually promote a mild hypertrophic response in aged mice (32 weeks). Another interesting aspect of *Pn*<sup>-/-</sup> mice is that they are less susceptible to fibrotic heart disease associated with long-term pressure overload or MI, resulting in better ventricular performance.<sup>15</sup> These results suggest that loss of periostin is protective to the heart long-term, provided the initial insult is survived.

An entirely novel function for periostin was recently reported whereby cardiomyocyte proliferation and the repair of injured myocardium was shown to be regulated by this protein.<sup>9</sup> In addition to reporting increased proliferation, Kühn et al showed that periostin expression prevented fibrosis and reduced cardiac hypertrophy after an injury event, results that are not consistent with other reports.<sup>15</sup> A potential reason for these differences may be that Kühn et al used a recombinant truncated form of periostin (amino acids 22 to 669 of 811) in ascertaining biological effects.<sup>9</sup> Using this abridged protein, as well as a full-length protein, the authors reported increased proliferation rates in adult rat cardiac myocytes cultured for a total of 12 days.<sup>9</sup> These latter results may also be influenced by the known process of dedifferentiation that occurs in adult myocytes cultured for this length of time. We failed to observe any induction of cell cycle activity in a model system that is known to be permissive to cell cycle reentry, neonatal myocytes in culture for 2 or 3 days.

More remarkably, Kühn et al reported that application of the truncated form of periostin with a gel foam patch onto infarcted rat hearts reduced fibrosis, reduced cardiac hypertrophy, increased myocyte proliferation, and improved cardiac function.<sup>9</sup> However, overexpression of full-length periostin in the mouse heart by transgenesis (via the well-characterized  $\alpha$ -myosin heavy chain promoter), which results in the proper accumulation of periostin protein in the ECM without detectable intracellular retention, did not increase myocyte number at baseline, nor did it augment indices associated with cardiac repair after MI injury.<sup>15</sup> However, 1 potential difference with our study is that the *Pn* transgene was used in a constitutive manner, so that periostin protein was always present, whereas Kühn et al applied recombinant periostin acutely. We could not mimic this acute affect because the inducible *Pn* transgene takes 4 to 6 weeks to achieve reasonable expression because of the time it takes doxycycline to clear the murine system. Also to be considered is that we did not observe differences in infarct size in any of the experimental or control groups, suggesting that the presence of the tetracycline transactivator was not a confounding variable (data not shown). Another potential difference is that we used the mouse, whereas Kühn et al used the rat for infarct studies. Finally, our transgenic mice expressed only 1 isoform of periostin, of which there are many alternately spliced versions present in the heart.<sup>15</sup> Thus, it is possible that another spliced form of periostin could have cell cycle promoting activity that is akin to the truncated version of periostin used by Kühn et al.<sup>9</sup>

Although our data are negative concerning a role for periostin in regulating myocyte proliferation, we believe that they are credible given the nature of the model systems used and our consistent use of positive and negative controls. First,

we carefully assessed the possibility that lack of periostin or constitutive expression of periostin throughout embryonic and postnatal development would alter myocyte numbers in the heart, although no effect was observed. By comparison, transgene mediated overexpression of calmodulin in the developing mouse heart promoted a 73% increase in myocyte number by embryonic day 19 and a 40% increase in myocyte number in the adult heart.<sup>32</sup> Overexpression of either insulin-like growth factor 1 in the heart or Bcl-2 increased myocyte number, as measured by assessment of myocyte size normalized to heart weight and volume fractions.<sup>33,34</sup> Overexpression of telomerase in the heart by transgenesis also produced greater myocyte content in the heart with some hypertrophy.<sup>35</sup> Second, neither *Pn*<sup>-/-</sup> mice nor periostin-overexpressing transgenic mice showed any difference in stimulated cell cycle activity in myocytes within the periinfarct zone of the adult mouse heart. Third, application of periostin by multiple methods was without effect on proliferation and cell cycle activity in cultured neonatal cardiomyocytes. Fourth, Shimazaki et al also noted no difference in Ki67-positive cells in the infarct border zone of *Pn*<sup>-/-</sup> mice.<sup>16</sup> In conclusion, although real differences in experimental conditions may have underlain an inability to detect proliferation and regeneration in our mice, we favor the alternate hypothesis that periostin mostly functions in collagen fibrillogenesis, ventricular remodeling, and facilitating cellular movements in areas of injury or ongoing epithelial-mesenchymal transition.

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## Disclosures

None.

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