Differential Behaviors of Atrial Versus Ventricular Fibroblasts
A Potential Role for Platelet-Derived Growth Factor in Atrial-Ventricular Remodeling Differences

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Background—In various heart disease paradigms, atria show stronger fibrotic responses than ventricles. The possibility that atrial and ventricular fibroblasts respond differentially to pathological stimuli has not been examined.

Methods and Results—We compared various morphological, secretory, and proliferative response indexes of canine atrial versus ventricular fibroblasts. Cultured atrial fibroblasts showed faster cell surface area increases, distinct morphology at confluence, and greater α-smooth muscle actin expression than ventricular fibroblasts. Atrial fibroblast proliferation ([^3]H]thymidine incorporation) responses were consistently greater for a range of growth factors, including fetal bovine serum, platelet-derived growth factor (PDGF), basic fibroblast growth factor, angiotensin II, endothelin-1, and transforming growth factor-β1. Normal atrial tissue showed larger myofibroblast density compared with ventricular tissue, and the difference was exaggerated by congestive heart failure. Congestive heart failure atria showed larger fractions of fibroblasts in mitotic phases compared with ventricles and displayed enhanced gene expression of fibroblast-selective markers (collagen-1, collagen-3, fibronectin-1). Gene microarrays revealed 225 differentially expressed transcript probe sets between paired atrial and ventricular fibroblast samples, including extracellular matrix (eg, fibronectin, laminin, fibulin), cell signaling (PDGF, PDGF receptor, angiopoietin, vascular endothelial growth factor), structure (keratin), and metabolism (xanthine dehydrogenase) genes, identifying PDGF as a candidate contributor to atrial-ventricular fibroblast differences. PDGF receptor gene expression was greater in normal atrium compared with ventricle, and congestive heart failure differentially enhanced atrial versus ventricular PDGF and PDGF receptor gene expression. PDGF receptor protein expression and α-smooth muscle actin protein expression were enhanced in isolated congestive heart failure fibroblasts. The PDGF receptor tyrosine kinase inhibitor AG1295 eliminated fetal bovine serum- and transforming growth factor-β1–stimulated atrial-ventricular fibroblast proliferative response differences.

Conclusions—Atrial fibroblasts behave differently than ventricular fibroblasts over a range of in vitro and in vivo paradigms, with atrial fibroblasts showing enhanced reactivity that may explain greater atrial fibrotic responses. PDGF signaling is particularly important for atrium-selective fibroblast responses and may represent a novel target for arrhythmogenic atrial structural remodeling prevention. (Circulation. 2008;117:1630-1641.)

Key Words: atrial fibrillation ■ extracellular matrix ■ fibrosis ■ heart failure ■ remodeling
of atrial and ventricular fibroblasts isolated from individual canine hearts, assessed their gene expression profiles, and evaluated their response to platelet-derived growth factor (PDGF) receptor (PDGFR) stimulation and inhibition. We subsequently sought evidence for corresponding differences in vivo.

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**Methods**

This section summarizes key methods in abbreviated form. A detailed description of all methods is found in the Methods section of the online-only Data Supplement.

**Cell Isolation, Culture, and Characterization**

Experiments were performed with cardiac fibroblasts isolated, cultured, and characterized as described previously from normal adult male 23- to 31-kg mongrel dogs (n=22). Preliminary studies revealed similar behavior in left atrial appendage- and left atrial free wall–derived fibroblasts (online-only Data Supplement Figure I). For practical purposes (tissue availability), further in vitro experiments used only first-passage cells from the left atrial appendage and left ventricular free wall. First-passage cells demonstrated >95% coexpression of α-smooth muscle actin (αSMA) and vimentin (online-only Data Supplement Figure II), indicating a myofibroblast phenotype resembling the activated cell type involved in remodeling and fibrosis. In all experiments, paired atrial and ventricular cell samples from each dog were plated in parallel at equal density, allowed to adhere for 24 hours, and then rendered quiescent in serum-free medium for 24 hours before treatment.

**Morphological Analysis**

Bright-field images of primary-passage atrial and ventricular fibroblasts (n=6 dogs per observation) were obtained after isolation (t=0) and at culture days 1, 2, 3, and 7 (at confluence). Duplicate random-field images were taken each day, coded, and quantified by an observer blinded to cell type. Cells were measured along their 2 longest perpendicular axes. The product of the long and short axes provided an index of surface area and the ratio of long to short axis, an index of cell morphology. Half-maximal growth times were quantified by nonlinear sigmoidal function regression.

**Western Blot Analysis**

First-passage atrial and ventricular fibroblasts (n=6 dogs per observation) in the exponential growth phase were plated at 150 cells per 1 mm². Proteins were extracted, quantified, and processed as previously described. Protein samples (10 μg per lane) were separated with 10% PAGE-SDS electrophoresis and then transferred to polyvinylidene difluoride membranes. Membranes were blocked and incubated with mouse anti-αSMA (1/20 000), rabbit anti-PDGFRα (1/1000), and mouse anti-GAPDH (1/5000) primary antibodies, followed by either anti-mouse (1/10 000) or anti-rabbit (1/10 000) horseradish peroxidase–conjugated secondary antibodies. Signals were detected with chemiluminescence and quantified by video densitometry. Protein band intensities are expressed relative to GAPDH.

Figure 1. Bright-field images of primary-passage ventricular (Vent; A through D) and atrial (At; E through H) fibroblasts (magnification ×10; bar=70 μm). I, Mean±SEM cellular surface area. T50 is half-maximal growth-time. n=6 pairs. **P<0.01, atrial vs ventricular values on the same day.
[3H]Thymidine Incorporation
Fibroblast proliferation was evaluated by [3H]thymidine uptake.9 Cells were plated at 50 cells per 1 mm² (n = 6 to 11 per experiment) and after adhesion and quiescence were stimulated for 24 hours with 7% fetal bovine serum (FBS), PDGF-AB (10 ng/mL), basic fibroblast growth factor (5 ng/mL), angiotensin II (100 nmol/L), endothelin-1 (10 nmol/L), or TGFβ1 (0.15 ng/mL). In 6 additional experiments, cells were treated for 24 hours with 7% FBS or TGFβ1 in the presence of PDGFR tyrosine kinase inhibitor AG1295 (10 μmol/L) or vehicle. Data are expressed as fold change relative to baseline.

Canine DNA Microarrays
Total RNA was isolated from first-passage cells (n = 5 per group) grown to confluence in 7% FBS.10 After RNA quality control, 3-μg samples were fragmented and hybridized to Affymetrix Canine_2 microarrays. Chips were stained, washed, and visualized on an Affymetrix GeneChip Scanner 3000. Interarray brightness differences were corrected with Invariant Set Normalization in dChip. The model-based expression index integrating each gene probe intensity into a representative gene expression value was then computed.11 Significance Analysis for Microarrays was applied to detect differentially expressed genes with q<5.

CHF and Sham Control Dogs
An additional 32 dogs (20 to 35 kg) received a right ventricular tachypacemaker,4,5,11 which was either programmed for 2-week ventricular-tachypacing at 240-bpm to induce CHF (n = 17) or left inactivated (control; n = 15). Paired atrial and ventricular tissue samples from control and CHF dogs were used for fibroblast isolation or snap-frozen and stored at −80°C for confocal imaging and real-time reverse-transcription polymerase chain reaction.

Confocal Imaging
Serial 14-μm cryosections were prepared from control and CHF (n = 3 dogs each) tissues as previously described.12 Slides were incubated with mouse anti-cSMA and goat anti-vimentin primary antibodies (both 1/200), followed by donkey anti-mouse AF555 and donkey anti-goat AF488 secondary antibodies (both 1/600), with parallel negative controls omitting primary antibodies. Slides were imaged in Z series every 0.25 μm with a Zeiss LSM-510 inverted confocal microscope. Z series images were deconvolved with Huygens Professional 3.0 software. Matched atrial and ventricular tissue analysis was performed at equal magnifications over equivalent tissue areas and thickness. αSMA-vimentin coexpression (red-green overlap) was quantified with a SigmaScan Pro v.4 (Systat Software Inc, Richmond, Calif).
**TaqMan Real-Time Reverse-Transcription Polymerase Chain Reaction**

Total RNA was extracted from control (n=5) and CHF (n=7) atrial and ventricular tissue samples. Real-time reverse-transcription polymerase chain reaction was performed as described previously. Fluorescence signals were detected in duplicate, normalized to 18S-ribosomal RNA, and quantified with MxPro quantitative polymerase chain reaction software (Stratagene Corp, La Jolla, Calif).

**Flow Cytometry**

Freshly isolated control (n=4) and CHF (n=4) atrial and ventricular fibroblasts were washed 8 to 10 times in PBS, strained, and then permeabilized/ixed in cold 75% ethanol. Samples were stored at −20°C until analysis. When pelleted, cells were resuspended and incubated (4°C for 30 minutes) in staining solution containing propidium iodide at ~1×10⁶ cells per mL. Propidium-iodide fluorescence was obtained with a FACScan and CellQuest Pro software (both from BD Bioscience, Franklin Lakes, NJ).

**Statistical Analysis**

Data are presented as mean±SEM. Atrial versus ventricular data were analyzed by paired t tests in the absence of a potentially interacting factor. Otherwise, 2-way repeated-measures ANOVA was applied with models using either 1 or 2 repeated factors (for details, see the online Methods). In the case of a significant interaction between factors, contrasts based on the global model within the other main factor (time [day 0, 1, 2, 3, and 7], group [control versus CHF], or treatment [vehicle versus AG1295]). Criteria for normal distribution of residuals were verified for all statistical models. A 2-tailed value of P<0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**In Vitro Analyses of Atrial-Ventricular Fibroblast Differences**

We first addressed differential characteristics of highly purified primary-passage atrial versus ventricular fibroblasts, a well-established preparation for studying fibroblast properties.9,10

**Cellular Organization and Morphology**

Bright-field microscopic images of primary-passage fibroblasts are shown in Figure 1A through 1H. Cells moved from an initial rounded morphology toward a more elongated phenotype. This transition occurred faster in atrial cells (significant interaction between cell type and time: F=17.22; dfn=4; dfd=20; P<0.0001). The results of blinded quantitative analysis are shown in Figure 1I. Half-maximal growth was reached in ~1.4 days by atrial cells compared with ~2.2 days for ventricular (P<0.01) (interaction between cell type and time: F=9.20; dfn=4; dfd=25; P=0.0001). At confluence (day 7), despite similar surface areas (1.54±0.10 for atrial versus 1.61±0.08 for ventricular; P=NS), primary-passage atrial cells displayed distinct organization and morphology (Figure 2A and 2B). Atrial fibroblasts showed more long-axis alignment and elongated morphologies (Figure 2C) with greater long-axis and lesser short-axis dimensions (Figure 2D).

**αSMA Expression**

Differentiating fibroblasts acquire a highly secretory myofibroblast phenotype characterized by αSMA expression, which correlates with increased secretion of profibrotic extracellular matrix components like collagen and fibronectin.13,14 We therefore compared αSMA expression in atrial and ventricular fibroblasts grown to confluence in 7% FBS. Figure 3A shows representative immunoblots with distinct bands corresponding to αSMA (~42 kDa) and GAPDH (~36 kDa). αSMA expression was significantly greater in atrial than ventricular fibroblasts (Figure 3B).

**Stimulus-Induced Proliferation Responses**

Both enhanced secretory activity of individual fibroblasts and fibroblast proliferation, which increases fibroblast number, can contribute to fibrosis. We therefore compared growth stimulus–induced proliferative responses of first-passage atrial and ventricular fibroblasts plated at equal density (Figure 4A through 4F). Basal [³H]thymidine uptake was indistinguishable in serum-free conditions (797±83 disintegrations per minute in ventricular versus 802±105 disintegrations per minute in atrial; P=NS); however, proliferation of atrial cells was consistently greater than that of matched ventricular fibroblasts when stimulated with a range of
growth factors, including FBS, PDGF, basic fibroblast growth factor, angiotensin II, endothelin-1, and TGFβ.

Differences in Gene Expression Profile of Atrial Versus Ventricular Fibroblasts

To assess gene expression differences, we performed transcriptome-wide microarray analyses of atrial and ventricular fibroblasts grown to confluence in 7% FBS. Statistically significant differential expression was noted for 225 probe sets. Of these, 60 sequences had no homology to known proteins, leaving 165 probe sets. In some cases, multiple probe sets corresponded to the same gene. The Table shows genes with consistent differential expression on each of several probe sets (82 probe sets accounting for 34 genes). The differentially expressed genes were implicated in important processes involved in remodeling, including extracellular matrix formation (fibronectin, laminin, fibulin, collagen triple-helix repeat containing 1), cell signaling (PDGFRα, secreted frizzle-related protein, vascular endothelial growth factor, angiopoietin, insulin-like growth factor binding protein), cell structure (keratin type 1), and metabolism (xanthine dehydrogenase).

We then cross-referenced each probe set showing a significant difference against the complete microarray library from Affymetrix to identify all available probe sets corresponding to each gene. Online-only Data Supplement Table I lists each gene with at least 1 significantly changed probe set, the mean relative atrial-ventricular expression for all significantly different corresponding probe sets, and the number of probe sets showing statistically significant changes per total number of probe sets for that gene. When all probe sets showed differences in the same direction (even though some may not have been statistically significant), no asterisk is shown (Probe set divergence is indicated by an asterisk). Online-only Data Supplement Table II provides detailed data for each gene with at least 1 significantly changed probe set. For 35 single statistically significant probe sets, no additional probe sets were in the complete Affymetrix chip library. For 28 probe sets, additional probe sets showed a similar fold change trend that did not reach statistical significance. The remaining 20 single-hit probe sets had at least 1 additional probe set with directionally different pairwise fold changes that could represent false-positive detections.

Atrial Versus Ventricular Fibroblast Properties In Vivo

Having found important differences between atrial and ventricular fibroblasts in vitro, we sought evidence for differential atrial-ventricular fibroblast behaviors in intact hearts.

Figure 4. [3H]Thymidine (mean±SEM) incorporation in first-passage atrial (At) and ventricular (Vent) fibroblasts under stimulation by 7%-FBS (A), PDGF-AB (10 ng/mL; B), basic fibroblast growth factor (bFGF; 5 ng/mL; C), angiotensin II (AngII; 100 nmol/L; D), endothelin-1 (ET-1; 10 nmol/L; E), and TGFβ1 (0.15 ng/mL; F). Dashed horizontal line indicates baseline (nonstimulated cells); n=8 to 11 observations for each experiment. *P<0.05; **P<0.01; ***P<0.001, At vs Vent.
Table. Genes From First-Passage Atrial and Ventricular Fibroblasts (n=5 Dogs) That Were Differentially Expressed on Each of Several Probe Sets

<table>
<thead>
<tr>
<th>Functional Class and Gene</th>
<th>Fold Change± SEM (At/Vent)</th>
<th>Probe Sets, n</th>
</tr>
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<tbody>
<tr>
<td>Extracellular matrix</td>
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<td></td>
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<tr>
<td>Collagen triple-helix repeat containing 1</td>
<td>1.80±0.05</td>
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</tr>
<tr>
<td>Fibronectin 1</td>
<td>3.21±0.42</td>
<td>5</td>
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<tr>
<td>Fibulin-1</td>
<td>1.91±0.07</td>
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</tr>
<tr>
<td>Fibulin-5</td>
<td>4.15±0.29</td>
<td>3</td>
</tr>
<tr>
<td>Laminin β-1</td>
<td>2.86±0.41</td>
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</tr>
<tr>
<td>Matrix Gla protein</td>
<td>2.73±0.26</td>
<td>2</td>
</tr>
<tr>
<td>Cell signaling</td>
<td></td>
<td></td>
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<tr>
<td>Angiopoietin-like 4 protein</td>
<td>0.54±0.02</td>
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</tr>
<tr>
<td>Cytoplasmic tyrosine protein kinase BMX</td>
<td>4.22±1.24</td>
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</tr>
<tr>
<td>Phosphatidic acid phosphatase type 2B</td>
<td>1.74±0.07</td>
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<tr>
<td>PDGFRα</td>
<td>3.38±1.15</td>
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</tr>
<tr>
<td>Regulator of G-protein signaling, 24 kDa</td>
<td>3.46±0.07</td>
<td>2</td>
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<tr>
<td>Serum/glucocorticoid regulated kinase</td>
<td>1.91±0.21</td>
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<tr>
<td>Vascular endothelial growth factor D</td>
<td>0.40±0.08</td>
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<tr>
<td>Metabolism</td>
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<tr>
<td>Cysteine dioxygenase type I</td>
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<tr>
<td>Glycosyltransferase 8</td>
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<tr>
<td>Peptidylglycine α-amidating monoxygenase</td>
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<td>Xanthine dehydrogenase/oxidase</td>
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<tr>
<td>Structural</td>
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<tr>
<td>Keratin type I</td>
<td>0.46±0.03</td>
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<tr>
<td>Tetranectin</td>
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<tr>
<td>Tetraspanin 12</td>
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<tr>
<td>Cell cycle and differentiation</td>
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<td>Four-and-a-half LIM domains 1</td>
<td>1.95±0.13</td>
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<td>Polo-like kinase 2</td>
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<td>Secreted frizzled-related protein 1</td>
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<td>CD59 antigen p18-20</td>
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<td>Sushi repeat-containing protein</td>
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<tr>
<td>Binding protein</td>
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<td>Insulin-like growth factor binding protein 6</td>
<td>2.38±0.16</td>
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<td>Secreted modular calcium-binding protein 1</td>
<td>5.97±3.68</td>
<td>2</td>
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<tr>
<td>Adhesion and migration</td>
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<tr>
<td>Dermatopontin</td>
<td>3.34±0.16</td>
<td>2</td>
</tr>
<tr>
<td>Tropomodulin 1</td>
<td>2.48±0.17</td>
<td>2</td>
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<tr>
<td>Protease and RNase/inhibitors</td>
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<tr>
<td>Protease inhibitor 7</td>
<td>4.26±0.17</td>
<td>3</td>
</tr>
<tr>
<td>Ribonuclease 4</td>
<td>2.25±0.37</td>
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<tr>
<td>Miscellaneous</td>
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<tr>
<td>Placenta-specific gene 8 protein (C15 protein)</td>
<td>5.44±2.34</td>
<td>3</td>
</tr>
<tr>
<td>Total=34</td>
<td>...</td>
<td>82</td>
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</table>

Myofibroblast Content

We first compared atrial and ventricular myofibroblast content with immunofluorescence labeling of αSMA (red) and vimentin (green) in control and CHF tissue sections. Representative images are shown in Figure 5A through 5D. αSMA staining and vimentin staining were observed in the interstitial regions between bundles of surrounding cardiomyocytes (unlabeled; visible in the overlaid phase-contrast images; online-only Data Supplement Figure III shows image without phase-contrast overlay), with more extensive staining and coexpression (yellow) in CHF. Both markers and their coexpression were more evident in atrial compared with ventricular tissue. Vimentin/αSMA coexpression (an index of myofibroblast content) was greater in atria than in ventricles for both control and CHF hearts, and CHF significantly increased atrial coexpression (Figure 5E). A statistically significant interaction was present between tissue type (atrium versus ventricle) and condition (control versus CHF) in determining coexpression (F=30.89; dfn=1; dfd=4; P=0.0051), indicating that tissue type is a determinant of the fibroblast response to CHF.

Mitotic Indexes in Freshly Separated Fibroblasts

We subsequently used flow cytometry to evaluate in vivo fibroblast proliferative indexes in freshly isolated control and CHF atrial and ventricular fibroblasts (Figure 5F). Online-only Data Supplement Figure IV shows examples of the dual-parameter dot plots used for gating (Figure IVA) and histogram analysis of DNA content in propidium-iodide–stained cells (Figure IVB). Cells derived from control atria and ventricles exhibited comparable numbers of dividing cells (in either cell cycle S or G2/M phase). CHF slightly increased ventricular fibroblast division rates but greatly increased atrial fibroblast division. The interaction between tissue type and condition was significant (F=40.43; dfn=1; dfd=6; P=0.0007), indicating that cardiac chamber is a determinant of the in vivo fibroblast proliferative response to CHF.

Fibroblast Secretory Gene Function

To assess in vivo fibroblast secretory gene function, control and CHF tissue samples were analyzed by real-time reverse-transcription polymerase chain reaction, choosing fibroblast-selective extracellular matrix genes Col1, FN1, and Col3. Hematoxylin and eosin staining of sections from control and CHF hearts showed increased αSMA (red) and vimentin (green) in fibroblasts in CHF compared with control hearts. The interaction between tissue type and chamber condition was significant (F=37.29; dfn=1; dfd=10; P=0.0001), indicating that cardiac chamber is a determinant of the in vivo fibroblast proliferative response to CHF.

Potential Role of PDGF in Differential Behaviors

Both PDGF and its α receptor were significantly more strongly expressed in atrial than ventricular fibroblasts (online-only Data Supplement Table I). PDGF modulates growth factors, and a tightly regulated autocrine PDGF loop is responsible for bimodal proliferation of connective tissue.
cells in response to TGFβ.

We therefore assessed the potential role of PDGF in differential atrial-ventricular fibroblast behaviors.

We first evaluated atrial-ventricular PDGF-B and PDGFα gene expression differences in intact tissue samples corresponding to the microarray findings for cultured fibroblasts. PDGF-B expression was not significantly different in control tissues but was differentially enhanced in CHF dog atria (Figure 6D). PDGFα expression was greater in atria than ventricles at baseline, and this difference was exaggerated by CHF (Figure 6E). A statistically significant interaction was observed between tissue origin and condition for both PDGF-B (F = 23.39; dfn = 1; dfd = 9; P = 0.0009) and PDGFα (F = 14.58; dfn = 1; dfd = 10; P = 0.0034) expression. We next confirmed differential atrial-ventricular protein expression of PDGFα and αSMA in isolated control and CHF fibroblasts (Figure 7). CHF further enhanced baseline atrial-ventricular differences in PDGFα (F = 28.61; dfn = 1; dfd = 10; P = 0.0003) and αSMA (F = 18.17; dfn = 1; dfd = 10; P = 0.0017) expression, consistent with whole-tissue mRNA data and supporting a role for PDGF signaling in CHF fibroblast responses.

We then addressed the potential functional role of differential atrial-ventricular PDGF by determining whether PDGF signaling is important for enhanced atrial fibroblast proliferation responses to other factors. Atrial and ventricular fibroblast [3H]thymidine incorporation was compared in response to 24-hour stimulation with FBS (Figure 8A) and TGFβ (Figure 8B) in the presence and absence of the PDGFR tyrosine kinase antagonist AG1295 (10 μmol/L). AG1295 significantly reduced the atrial and ventricular fibroblast proliferative responses induced by FBS and eliminated the differential atrial-ventricular fibroblast behavior. Similarly, the differential response of atrial fibroblasts to TGFβ stimulation was eliminated by AG1295. The interactions between cell type and PDGF-inhibiting effects were highly significant (F = 54.91; dfn = 1; dfd = 5; P = 0.0007 for FBS; and F = 60.83; dfn = 1; dfd = 5; P = 0.0006 for TGFβ). These data indicate that PDGF signaling is essential for the discernable atrial-ventricular fibroblast responses induced by FBS and TGFβ.

**Discussion**

We have demonstrated that despite overall phenotypic similarity, atrial fibroblasts are consistently more reactive than ventricular fibroblasts and show distinct growth characteristics, proliferative responses, and gene expression profiles. PDGF-related differences play an important role in atrial-ventricular fibroblast proliferative response discrepancies.

**Evidence for Differential Atrial-Ventricular Fibrotic Remodeling**

Cardiac chamber–specific gene expression patterns exist throughout development and occur in normal and...
diseased myocardium. Cardiac overexpression of junctin, an integral membrane protein colocalizing with ryanodine receptors and calsequestrin, causes markedly enlarged, severely fibrotic atria with much more limited ventricular fibrosis. Overexpression of the gene expression regulator cAMP response element modulator produces hypertrophic cardiomyopathy with markedly enlarged atria but not ventricles. Cardiac angiotensin-converting enzyme overexpression causes atrial dilation, fibrosis, and AF despite normal ventricular function and structure. Mice with constitutively active TGFβ show atrial fibrosis without changes in ventricular size, function, or structure. In all these genetically engineered mouse models, atrial fibrosis greatly exceeds ventricular fibrosis despite similar transgene expression in both regions. Some of these models (eg, angiotensin-converting enzyme overexpression) lack CHF, excluding differential atrial-ventricular hemodynamic stress.

Relation to Previous Studies

Our finding of greater myofibroblast content in normal atria versus ventricles agrees with observations of greater fibrous tissue and extracellular matrix volume in normal atria, previously hypothesized to be related to chamber-specific functional requirements, pressure, and volume. Larger myofibroblast content in CHF atria compared with ventricles agrees with previous findings of greater fibrosis in the atria versus ventricles of CHF dogs, particularly in light of data showing that increased fibroblast density correlates with myocardial fibrosis.

Our observations contribute to a growing literature on tissue-specific fibroblast diversity. Rat fibroblasts cultured from skeletal muscle, dermis, and lung show different patterns of αSMA expression, cytoskeletal pattern, and cell adhesion structures. Human fibroblasts cultured from proximal airways are distinct from peripheral lung parenchymal fibroblasts in size, morphology, and proliferative and secretory function. Discrete fibroblast populations in rat lung tissue maintain their characteristic features from the second through fourth passages. Differential responses to growth factors and gene expression patterns are seen in cultured human fibroblasts obtained from different periodontal regions. Cultured 10th-passage human skin fibroblasts from different anatomic sites display discrete gene expression patterns, with notable differences in genes implicated in extracellular matrix synthesis, lipid metabolism, and cell signaling. Our work shows that atrial and ventricular fibroblasts display many differences corresponding to previously observed variations in other tissue systems, including differences in morphology, secretory and proliferative function, response to growth factors, and gene expression. Our observations of in vivo cardiac systems and freshly isolated fibroblasts suggest that atrial-ventricular differences are particularly manifest in an important pathological condition (CHF) in which preferential atrial fibrosis is of pathophysiological relevance to a clinically important arrhythmia, AF.

Potential Mechanisms Underlying Atrial-Ventricular Fibroblast Differences

Cardiac fibroblasts derive from mesenchymal cells that originate in the proepicardial organ and produce chamber-specific cardiac development. Myocardial scar fibro-
blasts derive predominantly from resident intracardiac fibroblast populations\textsuperscript{37} and endothelial-to-mesenchymal transition,\textsuperscript{38} although there also is evidence for a contribution from bone marrow–derived cells.\textsuperscript{39} The atrial-ventricular fibroblast differences we observed may be due to intrinsic chamber-specific differences or to effects of the specific atrial hemodynamic, neurohumoral, and structural environment or their combination. We detected atrial-ventricular gene expression differences in important signaling molecules that could contribute to the functional differences we observed. PDGF appeared to play a particularly important role. PDGF, a regulatory peptide stimulated by tissue injury, activates PDGFR tyrosine kinase, initiating multiple secondary messenger signaling cascades,\textsuperscript{15,40} and synergizes with other growth factors, in particular TGF-\textit{\beta}1, in an autocrine and a paracrine fashion.\textsuperscript{16,41,42} TGF-\textit{\beta}1–induced proliferation in some systems requires the induction of PDGF in the presence of unmethylated PDGF DNA.\textsuperscript{42} Cardiac-restricted overexpression of either PDGF-C or PDGF-D isoforms cause fibrosis and dilated cardiomyopathy.\textsuperscript{43,44} PDGF is important in myofibroblast differentiation in vivo,\textsuperscript{45,46} alters fibroblast morphology, and induces collagen synthesis\textsuperscript{47} and proliferation\textsuperscript{48} in vitro. The elimination of atrial-ventricular fibroblast response differences to FBS and TGF-\textit{\beta}1 after treatment with AG1295 suggests that a substantial part of the differential cell type–specific response is mediated by PDGF, likely because of preferential atrial expression of both PDGF and its PDGFR.

**Novelty and Potential Significance**

To the best of our knowledge, this is the first direct comparison of atrial and ventricular fibroblasts and the first demonstration that they show systematic differences. These differences may contribute to the selective atrial fibrosis observed in various experimental models and to AF-promoting atrial fibrosis in humans. Furthermore, our observation that PDGF plays a central role in atrial-ventricular fibroblast differences provides insights into underlying molecular mechanisms. Our results may account (at least in part) for previously enigmatic findings such as the fact that mice expressing constitutively active TGF-\textit{\beta}1, with comparable atrial and ventricular TGF-\textit{\beta}1 activation, show substantial atrial fibrosis and an AF phenotype but little or no ventricular remodeling.\textsuperscript{6,7} Cardiac fibroblast function has been studied in detail but typically with the use of ventricular fibroblasts. Our findings indicate that caution may be needed in extrapolating the conclusions of studies performed with 1 cardiac fibroblast subtype to the other. Interventions to prevent atrial fibrosis are of potential therapeutic interest for AF.\textsuperscript{49} PDGF and associated signaling pathways may present new opportunities for preventing arrhythmogenic atrial structural remodeling.

**Potential Limitations**

Because [\textsuperscript{3}H]thymidine incorporation assays measure only 24 hours of stimulus-induced proliferation, our results relate to short-term cellular reactivity under conditions that cannot entirely model the complex in vivo milieu. We were...
are consistently upregulated in atrial cells versus ventricular. The microarray data do not address proteomic differences like posttranscriptional/translational modifications and regulatory changes and represent only a single time point snapshot of the continually changing transcriptome. We also considered only left atrial and ventricular fibroblasts and have not evaluated right-sided cells.

Much of our work was performed in first-passage cultured cardiac fibroblasts. Most previous studies of tissue- and organ-specific fibroblast properties were performed in cultured fibroblasts from the 2nd through 10th passages because of small cell numbers and incomplete purity in freshly isolated fibroblast samples. We cannot exclude the possibility that fibroblast properties were affected by the isolation and culture procedure. Our observations of greater numbers of myofibroblasts in atrial versus ventricular tissue samples with exaggeration in response to CHF, of greater fibroblast proliferative indexes in CHF atrium versus ventricle, and of differential atrial-ventricular expression of fibroblast-selective markers in snap-frozen tissue samples, particularly in CHF, all support the relevance of our in vitro findings to the in vivo context. We studied responses in 2-dimensional media, which may differ from behaviors in 3-dimensional matrixes. In addition, cocultures of myocytes, fibroblasts, and tissue sections were not used. Such systems might be very useful in determining interactions between the different cell types, which may reflect the in vivo situation even better.

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**Disclosures**

None.

**References**

6. Nakajima H, Nakajima HO, Salcher O, Dittie AS, Dembowsky K, Jing S, Field LJ. Atrial but not ventricular fibrosis in mice expressing a mutant...


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**CLINICAL PERSPECTIVE**

Cardiac tissue fibrosis is important in the progression of heart disease and plays an important role in cardiac arrhythmogenesis, particularly for atrial fibrillation. In a variety of cardiac disease models, atrial fibrosis is much more prominent than fibrosis in the ventricles, even when the profibrotic stimulus appears to be operating comparably at both the atrial and ventricular levels. This study examined the hypothesis that differences between atrial and ventricular fibroblast properties contribute to the predominant atrial fibrotic phenotype. To assess this possibility, we compared morphological, secretory, and proliferative responses of canine atrial versus ventricular fibroblasts. Atrial fibroblasts showed in vitro and in vivo behaviors indicating a greater tendency to activated myofibroblast dedifferentiation. Atrial fibroblast proliferation responses were consistently greater than ventricular responses for a variety of growth factors, including fetal bovine serum, platelet-derived growth factor, basic fibroblast growth factor, angiotensin II, endothelin-1, and transforming growth factor-β1. Atrial tissue showed larger myofibroblast density than ventricular tissue, particularly in the presence of congestive heart failure. Congestive heart failure atria had more active fibroblast division rates and enhanced gene expression of fibroblast-selective markers compared with ventricles. Gene microarrays revealed 225 differentially expressed transcript probe sets between paired atrial and ventricular fibroblast samples, including extracellular matrix, cell signaling, and metabolism genes, and identified platelet-derived growth factor as a potential contributor to atrial-ventricular fibroblast differences. Platelet-derived growth factor inhibition eliminated atrial-ventricular fibroblast proliferative response differences. Our results suggest that important differences exist in properties of atrial versus ventricular fibroblasts, that these differences contribute to atrium-selective fibrosis, and that platelet-derived growth factor signaling may be an interesting therapeutic target for the prevention of arrhythmogenic atrial structural remodeling.
Differential Behaviors of Atrial Versus Ventricular Fibroblasts: A Potential Role for Platelet-Derived Growth Factor in Atrial-Ventricular Remodeling Differences
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