Phenotype and Physiological Significance of the Endocardial Smooth Muscle Cells in Human Failing Hearts

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Background—Extravascular smooth muscle cells are often observed in the endocardium of human failing hearts. Here, we characterized the phenotype of those cells and investigated their physiological significance.

Methods and Results—We examined left ventricular biopsy specimens obtained from 44 patients with dilated cardiomyopathy and 6 nonfailing hearts. In Masson trichrome–stained histological preparations, bundles of smooth muscle cells were seen localized in the endocardium in 23 of the 44 specimens (none of the 6 controls). These cells were immunopositive for α-smooth muscle actin, type 2 smooth muscle myosin, desmin, and calponin, but were negative for embryonic smooth muscle myosin, vimentin, fibronectin, and periostin. This profile is indicative of a late differentiation (contractile) smooth muscle phenotype. Electron microscopy confirmed that phenotype, revealing the cells to contain abundant myofilaments with dense bodies but little rough endoplasmic reticulum or Golgi apparatus. In the endocardial smooth muscle–positive group, the left ventricular end-systolic volume index (73±34 versus 105±50 mL/m²; P=0.021), left ventricular peak wall stress (164±47 versus 196±43 dynes 10³/cm²; P=0.023), and left ventricular end-systolic meridional wall stress (97±38 versus 121±37 dynes 10³/cm²; P=0.036) were all significantly smaller, and the ejection fraction was larger (41±8.8 versus 33±9.3%; P=0.005) than in the endocardial smooth muscle–negative group. However, no histological parameters differed between the 2 groups.

Conclusions—Endocardial smooth muscle cell bundles in hearts with dilated cardiomyopathy exhibit a mature contractile phenotype and may play a compensatory role mitigating heart failure by reducing left ventricular wall stress and systolic dysfunction. (Circ Heart Fail. 2015;8:149-155. DOI: 10.1161/CIRCHEARTFAILURE.114.001746.)

Key Words: biopsy ■ cardiomyopathy, dilated ■ heart failure ■ myocytes, smooth muscle ■ ultrastructure

A layer of smooth muscle has been observed within the endocardium, which comprises the inner surface of the heart.1,2 Of the 2 distinct phenotypes exhibited by smooth muscle cells, synthetic and contractile,3,4 endocardial smooth muscle cells have been characterized as contractile, based on the expression of 2 smooth muscle myosin heavy chain isoforms.5-7 Okada8 used autopsied hearts to perform a clinico-pathological analysis of the thickening of the endocardium, and he found that while the thickness of the smooth muscle cell layer was <30 μm in normotensives, it could reach ≤200 μm in hypertensive patients with cardiac hypertrophy. This hyperplasia of the endocardial smooth muscle was most prominent at the outflow tract and in the papillary muscles of hearts subject to persistent hypertension. Similarly, in patients with dilated cardiomyopathy, the endocardial smooth muscle often reached a thickness of ≤80 μm. Within that context, it seems highly likely that endocardial smooth muscle plays a key role in the modification of cardiac function in various disease states.

Clinical Perspective on p 155

Although there have been attempts to characterize endocardial smooth muscle cells,5,9 their contribution to the pathophysicsiology of heart remains unclear. This, in part, is because of the fact that earlier studies used autopsy hearts, with which it is difficult to make a direct comparison between clinical and histological findings. However, it occurred to us that this limitation could be overcome by using endomyocardial biopsy specimens. In the present investigation, therefore, we studied the endocardial smooth muscle cells in biopsy specimens obtained from patients with hearts failure caused by dilated cardiomyopathy. We first used immunohistochemical
techniques and electron microscopy to characterize the phenotype of the endocardial smooth muscle cells and then assessed the relationship between the presence of the smooth muscle and selected hemodynamic and histological parameters.

Methods

Patient Profile
After obtaining approval for this study from our local ethics committee, patients with dilated cardiomyopathy were selected from among those who underwent left ventricular biopsy in Gifu University Hospital during the period from 2009 to 2013. All patients were evaluated clinically using both noninvasive and invasive methods. A diagnosis of dilated cardiomyopathy was made according to the definition and classification proposed by the World Health Organization—International Society and Federation of Cardiology task force,10,11 and a total of 44 patients were enrolled in the study, including 27 men and 17 women with a mean age of 59±13 (age range, 17–78) years. Patients with severe coronary artery stenosis (>75% luminal narrowing) and those with history of apparent hypertension were excluded from this study. All patients were given medications, including various combinations of a digitalis glycoside, diuretic, angiotensin-converting enzyme inhibitor, angiotensin II type 1 receptor blocker, β-blocker, and L-type calcium channel blocker. However, no drugs were given on the day of biopsy examination. The control group included 6 patients who had been clinically suspected of some cardiac disease because of chest pain, minimal electrocardiographic change, or arrhythmia, but for whom both noninvasive and invasive examinations of coronary angiography and biopsy findings were not diagnostic. The specimens were processed in the same way as those of the patients with dilated cardiomyopathy (DCM).

Echocardiographic, Hemodynamic, and Angiographic Evaluation
With all patients, 2-dimensional echocardiographic examinations were performed no >3 days before invasive examinations. The ventricular septal thickness and the left ventricular posterior wall thickness during the diastolic and systolic phases were recorded. All patients underwent right- and left-heart catheterization, biplane left ventriculography, and selective coronary angiography using standard techniques. The heart rate and pressures from the right and left heart were recorded, and the cardiac index was estimated using the thermodilution method. Peak and end-systolic meridional circumferential wall stresses were calculated as previously proposed.12,13 Left ventricular end-diastolic and end-systolic volume indexes and the ejection fraction were calculated from the left ventricular cineangiogram obtained in the right anterior oblique projection using Kennedy method.14

Endomyocardial Biopsy Procedure and Histological Evaluation
Biopsy specimens from the left ventricular free wall were obtained during the cardiac catheterization. One to 4 biopsy specimens were collected from each patient. One or 2 specimens were immediately fixed in a 10% buffered-formalin solution, dehydrated, embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin, Masson trichrome, or elastic van Gieson. Quantitative assessments, including cardiomyocyte size and fibrotic area, were performed in 20 randomly chosen high-power fields (<400) using a multipurpose color image processor (LUZEX F; Nireco, Kyoto, Japan). The cardiomyocyte size was expressed as the transverse diameter of cardiomyocytes cut at the level of the nucleus.15 The area of fibrosis was histologically determined in preparations stained with Masson trichrome and expressed as the % area of fibrosis in biopsy specimens. In addition, the mean numbers of inflammatory cells (total polymorphonuclear leukocytes, lymphocytes, and plasma cells) per high-power field were calculated. The % area of the endocardial smooth muscle in the subendocardium was measured by averaging the values of 3 Masson trichrome–stained sections cut at the different depth of a specimen; this was to mitigate in-specimen variation of smooth muscle distribution.

Immunohistochemistry
After deparaffinization, the 4-μm-thick sections were incubated with a primary antibody against α-smooth muscle actin (ab5694; Abcam and 1A4; DAKO), embryonic-type smooth muscle myosin (SMemb; ab684; Abcam), type 2 smooth muscle myosin (SM2; ab133567; Abcam), desmin (ab8592; Abcam), calponin (ab46794; Abcam), cardiac actin (23082-1-AP; Proteintech), α-smooth muscle actin (ab5694; Abcam), α-actinin (ab684; Abcam), cardiolipin (ab46794; Abcam), cardiac troponin (23082-1-AP; Proteintech), α-skeletal muscle actin (MUB0108P, Nordic-MUBio), vimentin (ab92547; Abcam), fibronectin (ab6328; Abcam, ab23750; Abcam), peristin (ab14041; Abcam), or tenasin (ab6346; Abcam). A Vectastain Elite ABC system (Vector Laboratories) was then used to immunostain the sections; diaminobenzidine served as the chromogen, and the nuclei were counterstained with hematoxylin. For double immunofluorescence, the sections incubated with primary antibodies were labeled with Alexa 488 (green) and Alexa 568 (red; both from Molecular Probes) and counterstained with Hoechst 33342 before being examined under a confocal microscope (C2; Nikon).
Electron Microscopy

Endomyocardial biopsy specimens other than those used for light microscopy were immediately fixed for 4 hours in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer. The specimens were then postfixed in 1% osmium tetroxide for 1 hour, dehydrated through graded ethanol and propylene oxide series, and embedded in Epon. Thereafter, they were thin-sectioned (80 nm) using an ultramicrotome, mounted on plain copper grids, stained with uranyl acetate and lead citrate, and examined by a Hitachi-700 electron microscope.

Statistical Analysis

Data were expressed as mean±SD. Statistical comparisons were made using Student t test and χ² analysis, when appropriate. Values of P<0.05 were considered significant.

Results

Clinical and Histological Findings

Table 1 summarizes the patients’ clinical, echocardiographic, hemodynamic, and angiographic data. The hearts of all 44 study participants had dilated left ventricular cavities: enlarged left ventricular end-diastolic and end-systolic volume index. The thickness of ventricular septum and left ventricular free wall at diastole was normal, but the left ventricular mass index was enlarged. Of the ventricular pressures, left ventricular end-diastolic pressure was normal, but the left ventricular mass index was enlarged. Among them, 5 contained endocardial smooth muscle cells (arrows in preparations stained with Masson trichrome) are positively stained for α-SMA, SM2, desmin, and calponin, but are negative for embryonic-type smooth muscle myosin (SMemb), α-cardiac actin, α-skeletal actin, vimentin, fibronectin, periostin, tenacin, and Sirius red. Bars, 100 μm. LM indicates light microscopy.

Immunohistochemical Findings

The endocardial bundles of smooth muscle cells observed in Masson trichrome–stained preparations were immunopositive for α-smooth muscle actin (Figure 1). On the contrary, immunostaining serial sections revealed the cells to be positive for SM2, desmin, and calponin, but negative for SMemb, α-cardiac actin, and α-skeletal actin. They thus display the late differentiation (contractile) phenotype of smooth muscle cells. Indeed, the labeling pattern of the endocardial smooth muscle cells was identical to that of vascular smooth muscle cells in the biopsy specimens (Figure 2). Those cells did not stain with Sirius red or immunostain for vimentin, fibronectin, periostin, or tenacin.

Electron Microscopic Findings

Ten specimens were available for electron microscopy. Among them, 5 contained endocardial smooth muscle cells forming bundles visible through the electron microscope. All of the smooth muscle cells were mature and exhibited the contractile phenotype. For example, their cytoplasm was tightly filled with thin filaments and numerous dense bodies, 2 specific structures characteristic of the smooth muscle contractile phenotype. By contrast, they contained only scarce synthetic organelles, such as Golgi apparatus and rough endoplasmic reticulum (Figure 3).
Relationships Between Smooth Muscle Cell Bundles and Clinicopathologic Parameters

When we compared the clinical and pathological parameters between the patients and specimens with and without endocardial smooth muscle cell bundles (Table 2; Figure 4A), we found that those with endocardial smooth muscle showed significantly less left ventricular peak wall stress (164±47 versus 196±43 dynes 10^3/cm^2; P=0.023) and left ventricular end-systolic meridional wall stress (97±38 versus 121±37 dynes 10^3/cm^2; P=0.036) than patients without endocardial smooth muscle. The left ventricular cavity at the end-systole was less dilated in the endocardial smooth muscle–positive group when compared with that in the negative group (73±34 versus 105±50 mL/m^2; P=0.021). In addition, the ejection fraction in the smooth muscle–positive group was significantly greater than in the negative group (41±8.8 versus 33±9.3%; P=0.005). All other clinical parameters did not significantly differ between the 2 groups. The % area of the endocardial smooth muscle showed a weak but significant correlation with the ejection fraction in patients with DCM (r=0.32; P=0.03; Figure 4B), but it did not with the other parameters including left ventricular wall stress and volume. It is assumed that the too large variation of the smooth muscle distribution prevented the % area of smooth muscle in a biopsy specimen from being sufficiently representative of that of the whole endocardium.

Discussion

The present study confirmed that endocardial smooth muscle cells in dilated cardiomyopathy exhibit the well-differentiated contractile phenotype at both the immunohistochemical and the ultrastructural levels. Moreover, we detected a noteworthy relationship between the presence of endocardial smooth muscle and several clinical parameters (ie, hearts that contained endocardial smooth muscle showed less left ventricular dilatation, less left ventricular wall stress, and better systolic function than hearts without endocardial smooth muscle).

Phenotype of the Endocardial Smooth Muscle Cells

In response to functional demands, vascular smooth muscle cells are able to modulate between synthetic and contractile phenotypes. Suzuki et al previously reported that endocardial smooth muscle cells in both normal and diseased hearts assume the well-differentiated contractile phenotype. However, that conclusion was based solely on the immunohistochemical detection of 2 smooth muscle myosin heavy chain isoforms. There are currently many antibodies available that can be used for phenotyping smooth muscle cells. Using those antibodies, we confirmed the earlier finding of Suzuki et al in DCM. More importantly, however, Suzuki et al used autopsied hearts, in

There was also no difference in the size of the cardiomyocytes or the degree of myocardial fibrosis and inflammatory cell infiltration between specimens with and without endocardial smooth muscle (Table 2).

![Figure 2](http://circheartfailure.ahajournals.org/)

![Figure 3](http://circheartfailure.ahajournals.org/)
which postmortem autolytic changes are unavoidable; ultrastructural examination, which is the gold standard for determining phenotype,3,4 was therefore not possible. Because we used biopsy specimens, we were able to do electron microscopic examinations, which revealed the contractile phenotype of the ultrastructure of endocardial smooth muscle cells.

Pathophysiological Function of the Endocardial Smooth Muscle

Wall stress is directly proportional to cavity diameter and inversely proportional to wall thickness (Laplace law).16 Consequently, wall stress and left ventricular remodeling (cavity dilatation and wall thinning) have a vicious relationship exacerbating one another to aggravate heart failure. In that context, the pathophysiological significance of the endocardial smooth muscle remains unclear. However, if one considers that the endocardial smooth muscle layer is thickened in failing hearts and that it is capable of tonic contraction, which would further thicken the ventricular wall, it seems plausible that this smooth muscle may modify cardiac function in a beneficial way. Consistent with that idea, we found that hearts with endocardial smooth muscle showed significantly less left ventricular peak and end-systolic meridional wall stress, as well as a significantly greater ejection fraction, than hearts without the smooth muscle.

Granulation tissue cells are known to disappear via apoptosis to form scar tissue in the postinfarction heart.17,18 When this apoptosis is inhibited by antiapoptotic treatment or by reperfusion of the infarct-related coronary artery, the adverse remodeling and dysfunction of the left ventricle are significantly attenuated.19–23 Preservation of granulation tissue containing vascular cells and myofibroblasts may lead to a thickening of the infarcted wall and a reduction in its circumferential length,

Table 2. Comparison of the Clinical and Histological Parameters Between Patients With DCM and Endocardial Smooth Muscle–Positive and Endocardial Smooth Muscle–Negative Biopsy Specimens

<table>
<thead>
<tr>
<th></th>
<th>Positive Specimens (n=23)</th>
<th>Negative Specimens (n=21)</th>
<th>P Value</th>
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<td>Clinical data</td>
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<tr>
<td>Age, y</td>
<td>58±15</td>
<td>53±13</td>
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<tr>
<td>Sex (M/F)</td>
<td>14/9</td>
<td>13/8</td>
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<td>LVDD, mm</td>
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<td>LVDS, mm</td>
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<tr>
<td>Heart rate, bpm</td>
<td>77±13</td>
<td>82±17</td>
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<td>LVSP, mmHg</td>
<td>141±30</td>
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<tr>
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<td>123±52</td>
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<tr>
<td>LVEVI, mL/m²</td>
<td>73±34</td>
<td>105±50</td>
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<td>LVF, %</td>
<td>41±8.8</td>
<td>33±9.3</td>
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<td>VSTd, mm</td>
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<td>LVPMWTd, mm</td>
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<td>LVMi, g/m²</td>
<td>147±42</td>
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<td>0.99</td>
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<tr>
<td>Speak, dynes 10³/cm²</td>
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<td>196±43</td>
<td>0.023*</td>
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<td>Sesm, dynes 10³/cm²</td>
<td>97±38</td>
<td>121±37</td>
<td>0.036*</td>
</tr>
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<td>Medication: digitalis</td>
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<td>9</td>
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<tr>
<td>Diuretics</td>
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<td>14</td>
<td>0.40</td>
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<tr>
<td>β-Blockers</td>
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<td>9</td>
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</tr>
<tr>
<td>ARB/ACEI</td>
<td>18</td>
<td>14</td>
<td>0.40</td>
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<tr>
<td>CCB</td>
<td>5</td>
<td>9</td>
<td>0.24</td>
</tr>
<tr>
<td>Histological data</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>% Area of SM</td>
<td>7.7±5.4</td>
<td>0±0</td>
<td>…</td>
</tr>
<tr>
<td>Cardiomyocyte size, µm</td>
<td>25±4.7</td>
<td>27±9.3</td>
<td>0.61</td>
</tr>
<tr>
<td>% Area of fibrosis,</td>
<td>30±16.5</td>
<td>34±13</td>
<td>0.45</td>
</tr>
<tr>
<td>Inflammatory cells (per HPF)</td>
<td>0.9±0.7</td>
<td>0.8±0.7</td>
<td>0.63</td>
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</table>

ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin II type 1 receptor blockers; CCB, calcium channel blockers; DCM, dilated cardiomyopathy; F, female; HPF, high-power field; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; LVEDVI, left ventricular end-diastolic volume index; LVEVI, left ventricular end-systolic volume index; LVMi, left ventricular mass index; LVPWTd, left ventricular posterior wall thickness at diastole; M, male; % Sesm, left ventricular end-systolic meridional wall stress; SMC area, % area of smooth muscle in the endocardium; and VSTd, ventricular septal thickness at end-diastole.

*Significant difference between the groups.

Figure 4. Comparison of the clinical parameters between patients with dilated cardiomyopathy (DCM) and endocardial smooth muscle–present and endocardial smooth muscle–absent biopsy specimens. A, Graphs comparing the left ventricular end-systolic volume index (LVESVI), ejection fraction (EF), left ventricular peak wall stress (Speak), and left ventricular end-systolic meridional wall stress (Sesm). B, Plots of correlation between the % area of endocardial smooth muscle and EF.
which would reduce wall stress, thereby mitigating the cardiac remodeling and dysfunction often seen at the chronic stage of myocardial infarction. The endocardial smooth muscle layers could contribute to the wall thickening, and tonic contraction of the smooth muscle could work toward shrinking the circumferential length of the left ventricular cavity. Both of those actions would reduce wall stress and thus attenuate heart failure. Therefore, we speculate that the endocardial smooth muscle plays a compensatory role against heart failure by diminishing left ventricular wall stress.

Yet unknown is the mechanism for appearance of smooth muscle cells in the endocardium of failing hearts. Stehbens et al. hypothesized that chronic hemodynamic overload–induced degenerative changes in the endocardium give rise to thickened endocardium consisting of collagen, elastin, and smooth muscle cells, which could be analogous to wound healing. Endocardial thickening is actually most prominent in disease states in which deterioration of cardiac function was most remarkable. However, smooth muscle cells present during wound repair generally display the synthetic phenotype, appearing inconsistent with our present finding. It is possible that those cells might have transdifferentiated into smooth muscle cells with contractile phenotype along with transition to chronic phase after cessation of focal inflammation. However, mechanisms of such transdifferentiation are also unknown because the phenotypic modulation of smooth muscle cells requires the coordinate regulation of several genes, which means that smooth muscle cell phenotype is likely governed by the activity of a transcription factor network.

Limitations

Although we speculated a compensatory role of the endocardial smooth muscle against heart failure based on its significant correlation with several clinicopathological parameters, we are aware that it is generally impossible to determine cause and effect relationship between structure and function in a human study. Because the sample size was modest in the present study, rates may be high for both types of statistical error (false-positive and false-negative). In addition, we cannot be certain that the biopsy specimens are representative of the entire ventricle. Consequently, the risk of sampling errors should be taken into account. It is noteworthy that although left ventricular contractility was better in the patients with smooth muscle–positive specimens, there was no significant difference in any histological parameters between specimens with and without endocardial smooth muscle. Such negative results may reflect substantial variation in the size of the cardiomyocytes within each small biopsy specimen.

Conclusions

Endocardial smooth muscle bundles in human hearts failing caused by DCM exhibit a well-differentiated contractile phenotype and may play a clinically compensatory role against heart failure by reducing left ventricular wall stress and systolic dysfunction.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Extravascular smooth muscle cells are often observed in the endocardium of human failing hearts. Here, we characterized the phenotype of those cells and investigated their physiological significance using left ventricular biopsy specimens obtained from 44 patients with dilated cardiomyopathy and 6 nonfailing hearts. Bundles of smooth muscle cells were seen localized in the endocardium in 23 of the 44 specimens (none of the 6 controls). We confirmed that endocardial smooth muscle cells in dilated cardiomyopathy exhibit the well-differentiated contractile phenotype at both the immunohistochemical and the ultrastructural levels. Moreover, we detected a noteworthy relationship between the presence of endocardial smooth muscle and several clinical parameters (ie, hearts that contained endocardial smooth muscle showed less left ventricular dilatation, less left ventricular wall stress, and better systolic function than hearts without endocardial smooth muscle). Endocardial smooth muscle cells may play a clinically compensatory role against heart failure by reducing left ventricular wall stress and systolic dysfunction.
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