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

Okada et al: Endocardial SMC in Failing Hearts

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Abstract

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’s 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 vs. 105±50 mL/m², p = 0.021), left ventricular peak wall stress (164±47 vs. 196±43 dynes·10⁻⁶/cm², p = 0.023) and left ventricular end-systolic meridional wall stress (97±38 vs. 121±37 dynes·10⁻⁶/cm², p = 0.036) were all significantly smaller and the ejection fraction was larger (41±8.8 vs. 33±9.3%, p = 0.005) than in the endocardial smooth muscle-negative group. However, no histological parameters differed between the two 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.

Key Words: dilated cardiomyopathy; biopsy; heart failure; smooth muscle cell; ultrastructure
A layer of smooth muscle has been observed within the endocardium, which comprises the inner surface of the heart.\textsuperscript{1,2} Of the two distinct phenotypes exhibited by smooth muscle cells, synthetic and contractile,\textsuperscript{3,4} endocardial smooth muscle cells have been characterized as contractile, based on the expression of two smooth muscle myosin heavy chain isoforms.\textsuperscript{5-7} Okada used autopsied hearts to perform a clinicopathological analysis of the thickening of the endocardium, and he found that while the thickness of the smooth muscle cell layer was less than 30 \textmu m in normotensives, it could reach up to 200 \textmu m in hypertensive patients with cardiac hypertrophy.\textsuperscript{8} 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 up to 80 \textmu m. 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.

Although there have been attempts to characterize endocardial smooth muscle cells,\textsuperscript{5,9} their contribution to the pathophysiology of heart remains unclear. This due in part to 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 employing 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 relation 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 committees, 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, 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 non-invasive 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 DCM.

Echocardiographic, Hemodynamic, and Angiographic Evaluation
With all patients, two-dimensional echocardiographic examinations were performed no more than 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 both 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. 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's method.
Endomyocardial Biopsy Procedure and Histologic Evaluation

Biopsy specimens from the left ventricular free wall were obtained during the cardiac catheterization. One to four biopsy specimens were collected from each patient. One or two 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's trichrome or elastic van Gieson. Quantitative assessments, including cardiomyocyte size and fibrotic area, were carried out in 20 randomly chosen high power fields (HPF, x400) 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's 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 HPF were calculated. The % area of the endocardial smooth muscle in the subendocardium was measured by averaging the values of 3 Masson's 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 (α-SMA; 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), α-cardiac actin (23082-1-AP, Proteintech), α-skeletal actin (MUB0108P, Nordic-MUBio), vimentin (ab92547, Abcam), fibronectin (ab6328, ab23750, Abcam), periostin (ab14041, Abcam) or tenacin (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, following incubation with primary antibodies the sections 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 h in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer. The specimens were then postfixed in 1% osmium tetroxide for 1 h, 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 means±SD. Statistical comparisons were made using Student's 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 elevated. Left ventricular systolic function was impaired, as indicated by the small ejection fraction. Both left ventricular peak systolic and end-systolic meridional wall stresses were elevated.

The biopsy specimens from DCM patients showed enlarged cardiomyocytes (Table 1), as well as fibrosis, myofiber disarray and inflammation to various extents. Staining the
preparations with Masson’s trichrome revealed bundles of smooth muscle cells within the endocardium in none of the 6 control specimens while 23 of the 44 biopsy specimens from DCM patients (0% vs. 52%, p = 0.025). We examined the Masson’s trichrome-stained sections obtained from 3 different depth of a specimen and indeed confirmed that all sections contained smooth muscle in the original smooth muscle-positive specimens while none did in the original smooth muscle-negative specimens. However, there was a large amount of in-specimen variability of the smooth muscle distribution. The % area of the endocardial smooth muscle in the subendocardium was 4.0±5.3% ranging from 0 to 25.3% in DCM patients.

Immunohistochemical Findings

The endocardial bundles of smooth muscle cells observed in Masson’s trichrome-stained preparations were immunopositive for α-SMA (Figure 1). On the other hand, immunostaining serial sections revealed the cells to be positive for SM2, desmin and calponin, but negative for SMemb, cardiac actin and 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, peristin or tenacin.

Electron Microscopic Findings

Ten specimens were available for electron microscopy. Among them, five 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, two specific structures characteristic of the smooth muscle contractile phenotype.3,4 By contrast, they contained only scarce synthetic organelles such as Golgi apparatus and rough endoplasmic reticulum (Figure 3).
Relations between Smooth Muscle Cell Bundles and Clinicopathologic Parameters

When we compared the clinical and pathological parameters between the patients/specimens with and without endocardial smooth muscle cell bundles (Table 2 and Figure 4A), we found that those with endocardial smooth muscle showed significantly less left ventricular peak wall stress (164±47 vs. 196±43 dynes-10^3/cm^2, p = 0.023) and left ventricular end-systolic meridional wall stress (97±38 vs. 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 compared with the negative group (73±34 vs. 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 vs. 33±9.3%, p = 0.005).

All other clinical parameters did not significantly differ between the two groups. The % area of the endocardial smooth muscle showed a weak but significant correlation with the ejection fraction in DCM patients (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.

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).

Discussion

The present study confirmed that endocardial smooth muscle cells in dilated cardiomyopathy exhibit the well-differentiated contractile phenotype at both the immunohistochemical and ultrastructural levels. Moreover, we detected a noteworthy relationship between the presence of endocardial smooth muscle and several clinical parameters; that is, 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.\(^3,4\) Suzuki et al.\(^5\) 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 two smooth muscle myosin heavy chain isoforms.\(^6,7\) There are currently a number of antibodies available that can be used for phenotyping smooth muscle cells. Using those antibodies, we confirmed the earlier finding of Suzuki et al. in dilated cardiomyopathy. More importantly, however, Suzuki et al. used autopsied hearts, in 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’s 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 anti-apoptotic treatment or by reperfusion of the infarct-related coronary artery, the adverse remodeling and dysfunction of
the left ventricle is significantly attenuated.\textsuperscript{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, which would reduce wall stress, thereby mitigating the cardiac remodeling and dysfunction often seen at the chronic stage of myocardial infarction.\textsuperscript{24-26} The endocardial smooth muscle layers could contribute to the wall thickening, and tonic contraction of the smooth muscle could work towards shrinking the circumferential length of the left ventricular cavity. Both of those actions would reduce wall stress and thus attenuate heart failure. We therefore 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.\textsuperscript{9} Endocardial thickening is actually most prominent in disease states in which deterioration of cardiac function was most remarkable.\textsuperscript{5,8} However, smooth muscle cells present during wound repair generally display the “synthetic” phenotype,\textsuperscript{3} 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 is also unknown because the phenotypic modulation of smooth muscle cells requires the coordinate regulation of a number of genes, which means that smooth muscle cell phenotype is very likely governed by the activity of a transcription factor network.\textsuperscript{27,28}

\textbf{Study 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.

Since 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 the size of the cardiomyocytes within each small biopsy specimen.

Conclusions

Endocardial smooth muscle bundles in human hearts failing due to dilated cardiomyopathy 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.

Acknowledgments

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Disclosures

None.
References


Table 1. Clinicopathological Data

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DCM, dilated cardiomyopathy; LVSP and LVEDP, left ventricular peak systolic and end-diastolic pressure; LVEDVI and LVESVI, left ventricular end-diastolic and end-systolic volume index; LVEF, left ventricular ejection fraction; VSTd and LVPWTd, ventricular septal and left ventricular posterior wall thickness at diastole; LVMI, left ventricular mass index; Speak, left ventricular peak wall stress; Sesm, left ventricular end-systolic meridional wall stress; ARB/ACEI, angiotensin II type 1 receptor blockers/angiotensin converting enzyme inhibitors; CCB, calcium channel blockers; %SMC area, %area of smooth muscle cells in the endocardium.
Table 2. Comparison of the Clinical and Histological Parameters Between DCM Patients with Endocardial Smooth Muscle-Positive and -Negative Biopsy Specimens

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LVSP and LVEDP, left ventricular peak systolic and end-diastolic pressure; LVEDVI and LVESVI, left ventricular end-diastolic and end-systolic volume index; LVEF, left ventricular ejection fraction; VSTd and LVVPWTD, ventricular septal and left ventricular posterior wall thickness at diastole; LVMI, left ventricular mass index; Speak, left ventricular peak wall stress; Sesm, left ventricular end-systolic meridional wall stress; ARB/ACEI, angiotensin II type 1 receptor blockers/angiotensin converting enzyme inhibitors; CCB, calcium channel blockers; %SMC area, %area of smooth muscle in the endocardium. *, a significant difference between the groups.
Figure Legends

Figure 1. Photomicrographs of histological and immunohistochemical preparations of left ventricular biopsy specimens. Immunopositive products are stained brown. Uppermost panels show preparations from a control non-DCM patient, stained with Masson’s trichrome (left panel) and immunostained for α-SMA (right panel), in which endocardial SMCs are absent. The lower panels show preparations obtained from DCM patients. The endocardial smooth muscle cells (arrows in preparations stained with Masson’s 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.

Figure 2. Confocal micrographs of immunohistochemical preparations of left ventricular biopsy specimens from DCM patients. The endocardial smooth muscle cells immunopositive for α-SMA are also immunopositive for SM2, calponin and desmin but immunonegative for SMemb. Bars, 20 μm.

Figure 3. Electron micrographs of the smooth muscle cells forming bundles within the endocardial extravascular area. (A) The ultrastructure of the smooth muscle cells is consistent with the contractile phenotype. (B) Highly magnified micrograph of the area in the square in the upper panel. Note the densely packed myofilaments and dense bodies (arrows) in the cytoplasm. Bars, 1 μm.

Figure 4. Comparison of the clinical parameters between DCM patients with endocardial smooth muscle-present and –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 ejection fraction.
Figure 2
Figure 3

A

Collagen

Nucl

B

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


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