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ORIGINAL RESEARCH ARTICLE

In situ nuclear DNA methylation in dilated cardiomyopathy: an endomyocardial biopsy study

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Abstract

Aims Although distinct DNA methylation patterns have been reported, its localization and roles remain to be defined in heart failure. We investigated the cellular and subcellular localization of DNA methylation and its pathophysiological significance in human failing hearts.

Methods and results Using left ventricular (LV) endomyocardial biopsy specimens from 75 patients with dilated cardiomyopathy (DCM; age: 58 ± 14 years old, %female: 32%) and 20 patients without heart failure (controls; age: 56 ± 17 years old, %female: 45%), we performed immunohistochemistry and immunoelectron microscopy for methylated DNA, 5-methylcytosine (5-mC). We next investigated possible relations of the incidence of 5-mC-positive (%5-mC$^+$) cardiomyocytes with clinicopathological parameters. Immunopositivity for 5-mC was detected in the cardiomyocytes and other cell types. The %5-mC$^+$ cardiomyocytes was significantly greater in DCM hearts than in controls (57 ± 13% in DCM vs. 25 ± 12% in controls, $P < 0.0001$). The localization of 5-mC immunopositivity in cardiomyocyte nuclei coincided well with that of heterochromatin, as confirmed by immunoelectron microscopy. Substantial DNA methylation was also observed in interstitial non-cardiomyocytes, but the incidences did not differ between control and DCM hearts (39 ± 7.9% in DCM vs. 41 ± 10% in controls, $P = 0.4099$). In DCM patients, the %5-mC$^+$ cardiomyocytes showed a significant inverse correlation with LV functional parameters such as heart rate ($r = 0.2391$, $P = 0.0388$), end-diastolic pressure ($r = 0.2397$, $P = 0.0397$), and ejection fraction ($r = -0.2917$, $P = 0.0111$) and a positive correlation with LV dilatation (volume index at diastole; $r = 0.2442$, $P = 0.0347$; and volume index at systole; $r = 0.3136$, $P = 0.0062$) and LV hypertrophy (mass index; $r = 0.2287$, $P = 0.0484$)—that is, LV remodelling parameters. No significant correlations between DNA methylation and the histological parameters of the biopsies, including cardiomyocyte hypertrophy, fibrosis, and inflammatory cell infiltration, were noted.

Conclusions The present study revealed increased nuclear DNA methylation in cardiomyocytes, but not other cell types, from DCM hearts, with predominant localization in the heterochromatin. Its significant relations with LV functional and remodelling parameters imply a pathophysiological significance of DNA methylation in heart failure.

Keywords Chromatin remodelling; Dilated cardiomyopathy; DNA methylation, Epigenetics

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Introduction

Clinical and experimental studies suggest epigenetic mechanisms play a pivotal role in the regulation of cardiac gene expression patterns and in the progression of cardiac hypertrophy and heart failure.[1–7] DNA methylation and histone modification are the most common mechanisms underlying epigenetic modulation of gene expression. It has been suggested that histone modification may contribute to the reprogramming of cardiac gene expression in heart failure,[1] but the role of DNA methylation is far less defined. Recent studies have shown that distinct epigenomic DNA methylation patterns are present at important DNA elements within the cardiac genome in human end-stage cardiomyopathy[2] and that differential DNA methylation correlates with differential expression of angiogenic factors in human heart failure.[3,4] Subsequent studies also using human cardiac tissue revealed differences in methylation of genes involved in pathways related to heart disease, which may provide an opportunity for differential diagnosis of heart failure.[5–8] Gilsbach et al.[9] provided information on how DNA methylation patterns change during developmental and pathological remodelling; interestingly, in a pressure overload model of heart failure, the DNMT3A-catalyzed patterns of DNA methylation detected in isolated cardiomyocytes were similar to those seen during development.

However, basic information such as cellular and subcellular localization of methylated DNA remains elusive. Because genome-wide studies using failing hearts analysed DNA in myocardial cells without distinguishing among cell types, it is impossible to know in which cell type(s), cardiomyocytes, or other interstitial cells, including fibroblasts, vascular cells, and inflammatory cells, undergo alterations in DNA methylation. It should be noted that non-cardiomyocytes, including interstitial and vascular cells account for 65–75% of the cells within the normal heart.[10–12] It will therefore be important to investigate the state of the DNA methylation in these cell types. In addition, nuclear structure is markedly altered in cardiomyocytes of failing hearts, characterized as being hypertrophied with severe crenation of nuclear membrane and widespread clumping of the chromatin, two hallmarks of nuclear hypertrophy.[13–16] We hypothesized that nuclear hypertrophy is associated with altered DNA methylation in cardiomyocyte nuclei in human failing hearts and may also be related to cardiac anatomical and functional alterations. To test that idea, we compared the DNA methylation levels and intranuclear localization of DNA methylation in endomyocardial biopsy specimens from human hearts with dilated cardiomyopathy (DCM) with non-failing hearts. And then, we investigated the possible association of DNA methylation with the haemodynamics and pathology of failing hearts with DCM.

Methods

The protocol of the present study was approved by the ethics committee of Gifu University Graduate School of Medicine (Approval No. 30-059). The investigation conformed to the principles outlined in the Declaration of Helsinki (*BMJ* 1964; ii: 177).

Patient profile

After obtaining approval for this study from our local ethics committees, patients with DCM were selected from among those who underwent left ventricular biopsy at Gifu University Hospital during the period from 2004 to 2013. All patients were evaluated clinically using both non-invasive and invasive methods. Diagnoses of DCM were made according to the definition and classification proposed by the World Health Organization-International Society and Federation of Cardiology task force.[17] A total of 75 patients were enrolled in the study, including 51 men and 24 women with a mean age of 58 ± 14 years (range: 17–78 years). Patients with severe coronary artery stenosis (>75% luminal narrowing) and those with a 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 collection. The control group without heart failure included 20 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 from patients with DCM.

Echocardiographic, haemodynamic, and angiographic evaluation

With all patients, two-dimensional echocardiographic examinations were performed no more than 3 days before invasive examinations using SSD-3500 (ALOKA, Tokyo, Japan) until March 2010 and iE33 (PHILIPS, Amsterdam, Netherlands) afterwards. The ventricular septal thickness and left ventricular (LV) posterior wall thickness were recorded during the diastolic and systolic phases. All patients underwent both right-heart and left-heart catheterization, biplane left ventriculography, and selective coronary angiography using standard techniques. The heart rate and arterial pressures from the right and left heart were recorded, and the cardiac index was estimated using the thermodilution method. Left ventricular end-diastolic and end-systolic volume indexes (LVEDVI

and LVESVI) and ejection fraction (LVEF) were calculated from the LV cineangiogram obtained in the right anterior oblique projection.

Endomyocardial biopsy procedure and histologic evaluation

From each patient, one to four biopsy specimens were collected from the left ventricular free wall during the cardiac catheterization. One or two specimens were immediately fixed in a 10% buffered-formalin solution, dehydrated, embedded in paraffin for light microscopy (Olympus BX53, Tokyo, Japan). In 4 μm thick paraffin sections stained with haematoxylin and eosin or Masson's trichrome, cardiomyocyte size (mean diameter of the transversely sectioned cells, 30 to 50 cardiomyocytes in random fields of view per section) and degree of fibrosis (from 0 to 3) were evaluated.[18] In addition, the mean numbers of inflammatory cells (total polymorphonuclear leukocytes, lymphocytes, and plasma cells) per high power field (×400) were calculated.

Immunohistochemistry

After deparaffinization, the 4 μm thick serial sections neighbouring the haematoxylin and eosin-stained ones were soaked in citrate buffer and heated for 10 min in a microwave oven for antigen retrieval and incubated with a primary antibody against 5-methylcytosine (5-mC; dilution 1/500, clone 33D3, Epigentek Group, Farmingdale, NY, USA). Acid denaturation was not performed to the tissue slides. A Vectastain Elite ABC system (Vector Laboratories, Burlingame, CA, USA) was then used to immunostain the sections; diaminobenzidine served as the chromogen, and the nuclei were counterstained with haematoxylin. The presence of immunoreactive 5-mC was assessed by light microscopic examination. Using all of the endomyocardial biopsy specimens, 5-mC in the nucleus was graded as positive or negative, and the percentage of 5-mC-immunopositive nuclei (%5-mC^+ nuclei) to a total of 30–50 cardiomyocyte nuclei in each specimen was calculated. Similarly, the %5-mC^+ nuclei were calculated in non-cardiomyocyte interstitial cell nuclei.

In order to specify cell types contained in the endomyocardial biopsy specimens, we performed immunohistochemical stain for dystrophin, a specific marker for skeletal muscle cells including cardiomyocytes, which makes it easy to distinguish cardiomyocytes from non-cardiomyocytes. After deparaffinization, the 4 μm thick sections were incubated with a primary antibody against dystrophin (dilution 1/100, ab15277, Abcam, Cambridge, UK). Antigen retrieval was not performed. A Vectastain Elite ABC system was then used to immunostain the sections; diaminobenzidine served as the chromogen, and the nuclei were counterstained with haematoxylin. Searching high power fields, we determined percentage of the cardiomyocyte population in specimens from six pairs of age-matched and gender-matched patients from the control and DCM groups.

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 post-fixed in 1% osmium tetroxide for 1 h, dehydrated through graded ethanol and propylene oxide series, and embedded in Epon. Thereafter, the specimens were thin-sectioned (70 nm) using an ultramicrotome, mounted on plain nickel grids, stained with uranyl acetate and lead citrate, and examined with an electron microscope (HT7700, Hitachi, Tokyo, Japan).

In the endomyocardial specimens, cell types were identified by their specific ultrastructure and/or position: cardiomyocytes by the presence of sarcomeres; endothelial cells by the innermost layer position of the vessels; fibroblasts by the spindle shaped cells containing rough endoplasmic reticulum, scattering in the interstitium; vascular smooth muscle cells by the wrapping position around the vascular endothelial cells of small arteries, containing dense actin filaments in the cutoplasm; pericytes by the wrapping position around the vessels; macropahges by the amorphous shape having pseudopods and containing foreign bodies, scattering in the interstitium; and polymorphonuclear cells by the segmented nucleus and cytoplasmic granules.

Immunoelectron microscopy

Thin sections (70 nm) were mounted on bare 200-mesh nickel grids. The methylated DNA was labelled using a mouse anti-5-mC antibody at a dilution of 1:100 in 2% horse serum-containing PB for 1 h at room temperature. The grids were then incubated with colloidal gold (particle size: 15 nm)–conjugated goat anti-mouse IgG (GAF-011-15, EY Laboratories, San Mateo, CA) at a dilution of 1:5 in 2% horse serum-containing phosphate buffer for 1 h at room temperature. After rinsing the grids with distilled water, they were counterstained with lead citrate and examined using an electron microscope. The grids were washed with phosphate buffer between each step. We checked the validity of this method by omitting the primary antibody (negative control).

Localization of immunogold-labelled 5-mC within cardiomyocyte nuclei was determined in 30 nuclei per specimen using specimens from six pairs of age-matched and gender-matched patients from the control and DCM groups: the density of the immunogold particles (per μm^2) was calculated manually per micrograph, as were

the fractions of immunogold particles localized in the heterochromatin and euchromatin within the nuclei. We similarly determined the density of nuclear immunogold particles labelling 5-mC in vascular endothelial cells and myocardial fibroblasts from those patients; 20 cells each were counted per specimen.

Statistical analysis

All statistical analyses were performed using Graph Pad PRISM Version 6.0 statistical software (GraphPad Software, CA). Data are expressed as means ± SD. Group comparisons were made using Student's *t*-tests. Spearman's analysis was used to determine correlations between parameters. Values of $P < 0.05$ were considered significant.

Results

Clinical and histological findings

Table 1 summarizes the patients' clinical, echocardiographic, haemodynamic, and angiographic findings. The hearts of all 75 study participants had dilated LV cavities characterized by enlarged left ventricles (i.e. increased LVEDVI and LVESVI) or hypertrophied left ventricles (i.e. increased LV mass index). In addition, LV end-diastolic pressure was elevated, and LV systolic function was impaired, as indicated by the small LVEF (38 ± 10% in DCM vs. 64 ± 6% in controls) and increased heart rate. The biopsy specimens from patients with DCM showed enlarged cardiomyocytes and increased fibrosis, varying degrees of inflammatory cell infiltration (*Table 1*). Cardiomyocyte population was 33 ± 5.3% in the control hearts and 23 ± 3.6% in DCM hearts ($n = 6$ each, $P = 0.0056$) (*Figures 1A* and *1B*), being compatible with earlier reports.[10-12]

DNA methylation in cardiac cells

All specimens positively immunostained for 5-mC, which was exclusively confined to the nuclei in both cardiomyocytes and non-cardiomyocytes (*Figures 1C* to *1D'*). In cardiomyocytes, the incidence of 5-mC-immunopositive (5-mC$^+$) nuclei was significantly greater in DCM patients (57 ± 13%, range: 24–95%) than in controls (25 ± 12%, range: 1.9–44%, $P < 0.0001$) (*Figure 1E*). In non-cardiomyocytes, on the other hand, the incidences were not different between the two groups (39 ± 7.9% in controls vs. 41 ± 10% in DCM, $P = 0.4099$) (*Figure 1F*).

Nuclear localization of methylated DNA and its relation to chromatin remodelling

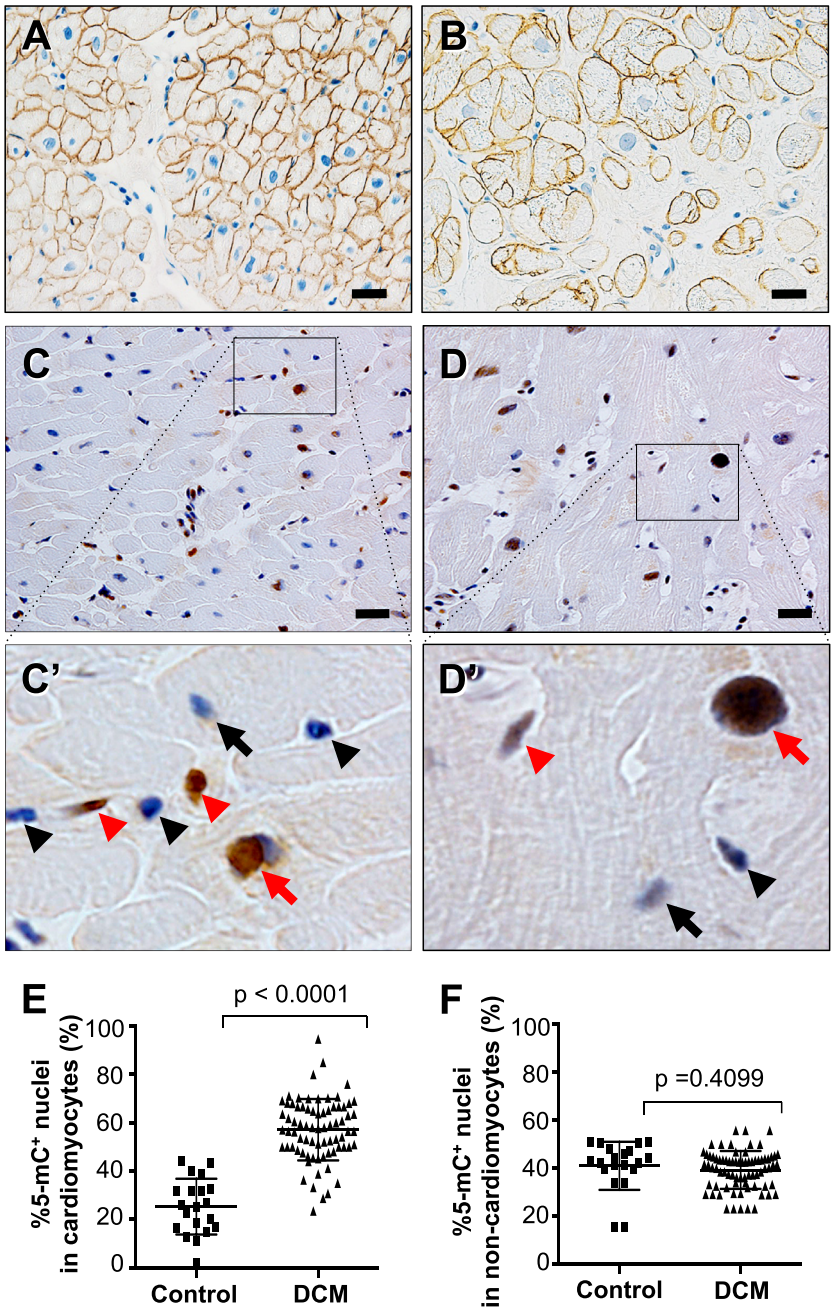
Cardiomyocyte nuclei in specimens from DCM patients generally exhibited degenerative changes whereby they were hypertrophied or bizarrely shaped and contained abundant heterochromatin.[13–16] To evaluate the spatial relationship between the heterochromatin and methylated DNA, we performed an immunoelectron microscopic analysis of the 5-mC. Immunogold particles bound to 5-mC concentrated predominantly on electron dense heterochromatin; electron lucent euchromatin was rarely stained (*Figure 2*). Quantitative analysis further confirmed that immunogold particles

Table 1 Clinicopathological data

	Non-failing hearts ($n = 20$)		DCM ($n = 75$)			
					Correlation with %5-mC$^+$ cardiomyocyte	
	Mean ± SD or *n*	Limits	Mean ± SD or *n*	Limits	*r* value	*P* value
Clinical data						
Age (year old)	56 ± 17	14–77	58 ± 14	17-81	−0.0661	0.5730
Gender (M/F)	11/9	—	51/24	—	—	—
LVSP (mm Hg)	137 ± 29	100–200	135 ± 25	75–200	−0.0314	0.7892
LVEDP (mm Hg)	13 ± 5.9	4–25	16 ± 8.2	4–40	0.2397	0.0397
Heart rate (bpm)	68 ± 13	47–94	77 ± 16*	53–132	0.2391	0.0388
LVEF (%)	64 ± 6	55–76	38 ± 10*	10–54	−0.2917	0.0111
LVEDVI (ml/m^2)	90 ± 19	57–135	124 ± 39*	30–222	0.2442	0.0347
LVESVI (ml/m^2)	32 ± 10	12–57	78 ± 33*	15–169	0.3136	0.0062
LVMI (g/m^2)	101 ± 42	57–206	137 ± 41*	65–248	0.2287	0.0484
Histological data						
Cardiomyocyte size (μm)	17 ± 2.6	12.8–21.8	26 ± 4.7*	13.9–40.3	0.0248	0.8325
Degree of fibrosis (0–3)	1.0 ± 0.7	0–2	1.7 ± 0.7*	0.5–3	0.0296	0.8009
Inflammatory cells (per HPF)	0.4 ± 0.5	0–1	0.9 ± 0.6*	0–2	0.0513	0.6621
Cardiomyocyte population (%)[a]	33 ± 5.3	24–38	23 ± 3.6*	18–28	—	—

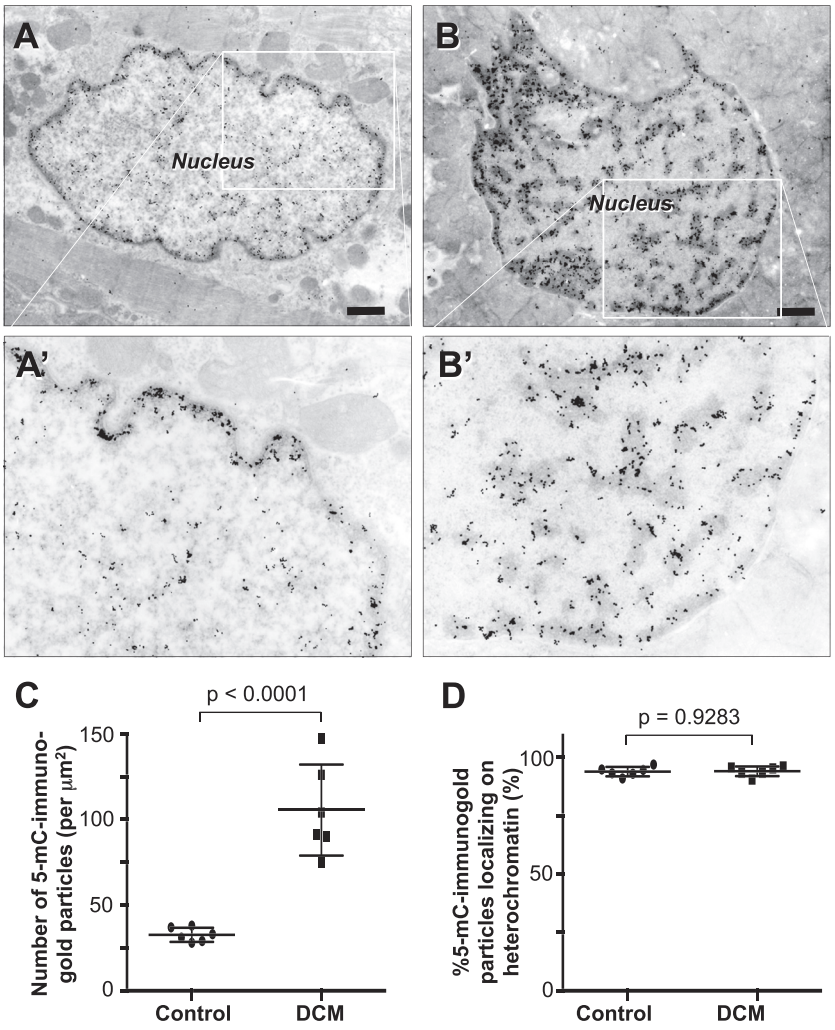
LVSP and LVEDP, left ventricular peak systolic and end-diastolic pressure; LVEDVI and LVESVI, left ventricular end-diastolic and end-systolic volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; HPF, high power field.
*$P < 0.05$ vs. non-failing hearts.
[a]$n = 6$ in each group.

Figure 1 Cell type specification and immunohistochemical detection of 5-mC in endomyocardial biopsy specimens at the light microscopic level. (A and B) Immunohistochemistry for dystrophin in a control specimen in panel A and a DCM specimen in panel B. Plasma membranes and T-tubules are stained specifically in cardiomyocytes. Scale bars, 20 μm. (C and D) 5-mC-positive nuclei are stained brown while 5-mC-negative nuclei are stained blue in both a control specimen in panel C and a DCM specimen in panel D. Panels A′ and B′ are highly magnified images of the boxed areas in panels A and B, respectively. Red arrows, 5-mC-positive cardiomyocyte nuclei; black arrows, 5-mC-negative cardiomyocyte nuclei; red arrowheads, 5-mC-positive non-cardiomyocyte nuclei; black arrowheads, 5-mC-negative non-cardiomyocyte nuclei. Scale bars, 20 μm. (E) Graph showing comparison of the % 5-mC-positive nuclei in cardiomyocytes between controls (n = 20) and DCM patients (n = 75). (F) Graph showing comparison of the %5-mC-positive nuclei in non-cardiomyocyte interstitial cells between controls (n = 20) and DCM patients (n = 75).



bound to 5-mC were more densely concentrated within cardiomyocyte nuclei affected by DCM (106 ± 27 particles/μm^2) than within control nuclei (33 ± 4 particles/μm^2, $P < 0.0001$). Moreover, the particles localized almost entirely in the heterochromatin; approximately 94% of the immunogold particles were localized on heterochromatin in both controls (94 ± 2.0%) and DCM (94 ± 2.1%).

Figure 2 Immunocytochemical detection of 5-mC in cardiomyocytes in endomyocardial biopsy specimens at the electron microscopic level. Immunogold particles bound to 5-mC localize predominantly in heterochromatin in both normal (A) and bizarrely shaped (B) nuclei. The bizarrely shaped nucleus is rich in heterochromatin where immunogold particles are abundant. Panels A′ and B′ are highly magnified and lightly printed images of the boxed areas in panels A and B, respectively. They highlight the relationship between heterochromatin and immunogold particles. Scale bars, 1 μm. (C) Graph showing comparison of the number of 5-mC bound immunogold particles between controls (n = 6) and DCM patients (n = 6). (D) Graph showing fraction of the 5-mC bound immunogold particles localized on heterochromatin in controls (n = 6) and DCM patients (n = 6).

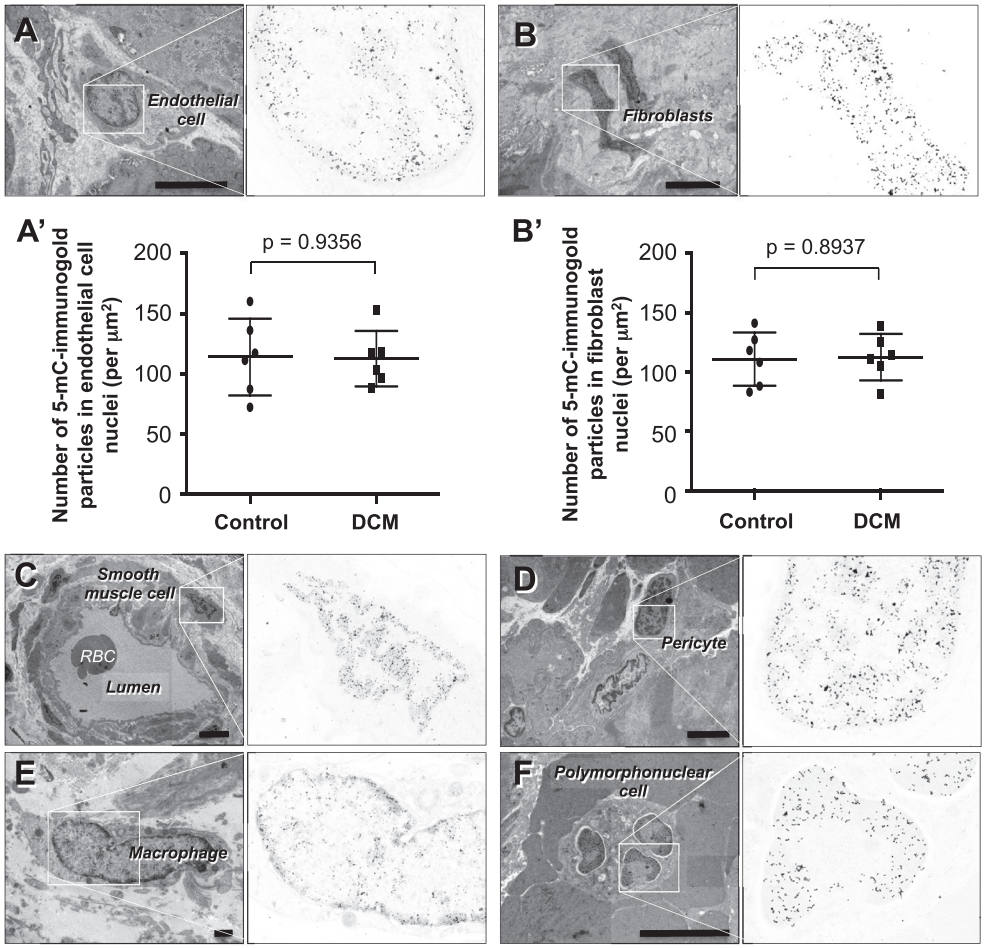


Immunoelecron microscopy confirmed the accumulation of immunogold particles bound to 5-mC in nuclear heterochromatin in all types of non-cardiomyocytes, including vascular endothelial cells, vascular pericytes, vascular smooth muscle cells, fibroblasts, macrophages, and circulating leukocytes (*Figure 3*). However, no apparent differences in the nuclear density of immunogold particles were noted among the different cell types between DCM and controls. Quantitative analysis revealed no difference between specimens from control and DCM hearts with respect to the number of immunogold particles within the nuclei of vascular endothelial cells (controls, 114 ± 32 particles/μm^2 vs. DCM, 113 ± 23 particles/μm^2, P = 0.9356) or myocardial fibroblasts (controls, 111 ± 23 particles/μm^2 vs. DCM, 113 ± 20 particles/μm^2, P = 0.8937).

Relations between methylated DNA and clinicopathological parameters

We investigated the relations between DNA methylation in cardiomyocyte nuclei and several clinicopathological parameters (age, gender, cardiac structure and function, and cardiac histology) in the DCM patients. There was no difference between male and female in the degree of DNA methylation in DCM patients (male: 57 ± 1.8% vs. female: 57 ± 2.5%, P = 0.7785). On the other hand, we found that there was a significant positive correlation between the percent 5-mC-positive cardiomyocytes and heart rate (*Table 1* and *Figure 4*). We also found a significant positive correlation between the percent 5-mC-positive cardiomyocytes and LV remodelling parameters, including

Figure 3 Immunocytochemical detection of 5-mC in non-cardiomyocyte interstitial cells in endomyocardial biopsy specimens from patients with DCM. (A) Capillary endothelial cell. (B) Fibroblast. (A′ and B′) Graphs showing comparison of the number of 5-mC bound immunogold particles in capillary endothelial cell nuclei (A′) and in fibroblast nuclei (B′) between controls (n = 6) and DCM patients (n = 6). Group comparisons were made using Student's t-tests. (C) Smooth muscle cell in a small artery. (D) Pericyte in an arteriole. (E) Macrophage. (F) Polymorphonuclear cell within a vascular lumen. Scale bars, 1 μm.



LV dilatation (LVEDVI and LVESVI) and LV hypertrophy (LV mass index). There was an inverse correlation with LV systolic function (LVEF). No significant correlations between DNA methylation and the histological parameters of the biopsies, including cardiomyocyte hypertrophy, fibrosis, and inflammatory cell infiltration, were noted (*Table 1*). In the control hearts, no significant correlation was noted between the degree of DNA methylation and any clinicopathological parameters ($P > 0.05$ in all).

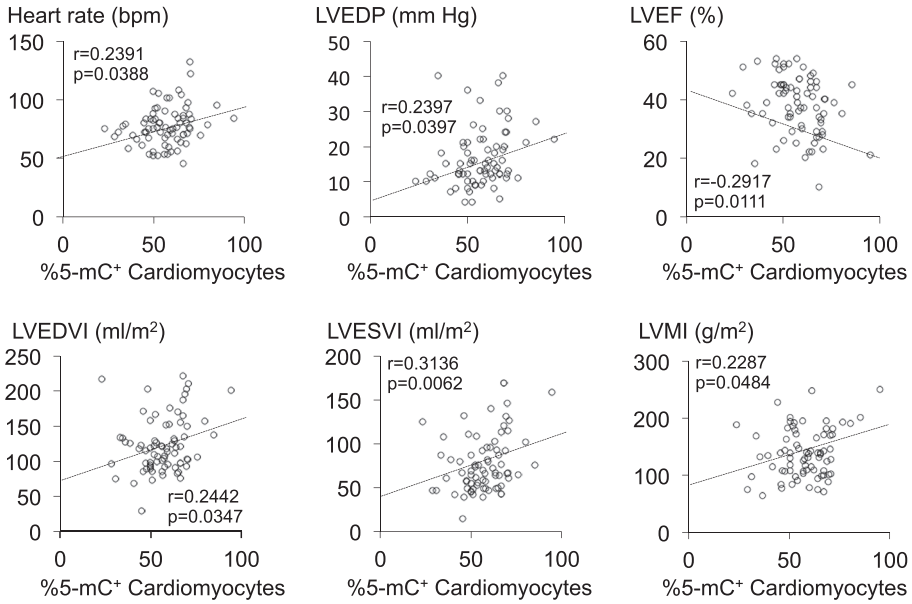
Discussion

The major findings of the present study of human endomyocardial biopsy specimens are the following: (i) nuclear DNA methylation detected through immunohistochemical labelling of 5-mC was increased in the failing hearts of patients with DCM, but the increase in nuclear DNA methylation was noted only in cardiomyocytes but not in other cell types; (ii) visualized at the subcellular level, 5-mC localized to heterochromatin; and (iii) DNA methylation was related to haemodynamic parameters associated with LV remodelling and LV dysfunction.

Increased DNA methylation in the cardiomyocytes from failing hearts

DNA methylation and histone modifications are the most common mechanisms of epigenetic modulation of gene expression. It has been suggested that histone modification may contribute to the reprogramming of cardiac gene expression in heart failure,[9,19] but the role of DNA methylation is far

Figure 4 Plots showing the correlations between the percent 5-mC-positive (%5-mC$^+$) cardiomyocytes and the indicated haemodynamic parameters in patients with DCM (n = 75 in each graph). LVEDP, LV end-diastolic pressure; LVEF, LV ejection fraction; LVEDVI, LV end-diastolic volume index; LVESVI, LV end-systolic volume index; LVMI, LV mass index.



less well defined. Several studies have investigated genome-wide DNA methylation in failing hearts. Movassagh et al. reported that DNA methylation is altered in human failing hearts, and this was confirmed in subsequent studies.[4–8] However, all of those studies assessed DNA methylation biochemically using immunoprecipitation chips, where DNA from not only cardiomyocytes but also other cell types within the myocardium was analysed without distinguishing among them. In the present study, we assessed DNA methylation through immunohistochemical staining for 5-mC, which enabled us to selectively evaluate DNA methylation at each cell type within the heart. Our findings indicate that cardiomyocyte-specific alteration (enhancement) of nuclear DNA methylation occurs in the hearts of DCM patients. No change in DNA methylation was noted in non-cardiomyocyte interstitial cells. It is somewhat unexpected that DNA methylation status was unchanged in cardiac fibroblasts from DCM patients, as it was previously suggested that fibroblasts are activated through modification of DNA methylation in response to transforming growth factor-β1 or hypoxia, both of which play roles within failing hearts.[20,21]

DNA methylation and chromatin remodelling

Cardiac myocytes display dramatic alterations of their phenotype in human hypertrophic and failing hearts. For example, the cytoplasm shows an increased subcellular organelle fraction accompanied by a decreased myofilament fraction, mitochondriosis, deposition of glycogen granules, and intracytoplasmic vacuoles.[13] Nuclear structure is also markedly altered and is characterized as being 'hypertrophied', with severe crenation of the nuclear membrane (bizarre shape) and widespread clumping of the chromatin, two hallmarks of 'nuclear hypertrophy'.[14–16] The molecular biological alterations within the hypertrophied nuclei in human failing hearts are largely unknown, though the nuclear structural changes are reminiscent of chromatin remodelling. The widespread chromatin clumping cytologically resembles heterochromatin, which appears as small, darkly staining, irregular particles scattered throughout the nucleus or accumulated adjacent to the nuclear envelope.[22] Because chromatin remodelling is one phenotype of epigenetic modification, epigenetic mechanisms are supposed to be significantly modified in hypertrophied nuclei within cardiomyocytes.

We examined the distribution of DNA methylation within nuclei through immunoelectron microscopic detection of 5-mC, which revealed that 5-mC-immunopositive particles gathered specifically in nuclear heterochromatin in both cardiomyocytes and interstitial non-cardiomyocytes. Heterochromatin is also associated with the di-methylation and tri-methylation of H3K9 in certain portions of the genome.[23] In addition, a special coincidence between DNA methylation and chromatin condensed as a result of its remodelling has been suggested.[24] Lamin A/C, a nuclear membrane protein, interacts with genome through lamin-associated domains and regulates gene expression, which is known to influence spatial reorganization of the chromatin.[25,26] A recent study

revealed rearrangement of lamin A/C-chromatin interaction and association of lamin-associated domain with increased DNA methylation in DCM.[27] Our findings seem to be consistent with those ideas.

Pathophysiological significance of DNA methylation in heart failure

Abnormal regulation of genes and/or proteins involved in sarcomere organization, expression of cardiac hormones and proto-oncogenes, and extracellular matrix remodelling that directly influences cardiac function and structure has been described in numerous studies of DCM and heart failure.[24,28–30] Moreover, it was recently suggested that epigenetic mechanisms play a pivotal role in the regulation of cardiac gene expression patterns during the progression of cardiac hypertrophy and failure.[1–8] In the present study, nuclear DNA methylation in cardiomyocytes was found to correlate significantly with parameters of LV remodelling (LV dilatation and LV hypertrophy) and LV dysfunction. There may thus be a significant association between nuclear DNA methylation in cardiomyocytes and the pathophysiology of heart failure.

However, recent studies have demonstrated the therapeutic potential of inhibiting DNA methylation in models of cardiac hypertrophy and reduced cardiac contractility. For example, treatment with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine blunted hypertrophic growth in a model of norepinephrine-induced hypertrophy;[31] while the inhibitor 5-azacytidine exerted antifibrotic and antihypertrophic effects in a spontaneously hypertensive rat model.[32] In addition, treatment with 5-aza-2′-deoxycytidine ameliorated cellular autophagy failure in Danon disease with progressive fatal cardiomyopathy by reactivating the silent LAMP2 gene allele.[33] Thus far, however, the link between DNA methylation and heart failure remains correlative. Additional studies will be needed to determine whether altered DNA methylation is causative and/or consequential, which will be essential for their development as therapeutic targets in the treatment of heart failure.[2]

Study limitations

It is generally not possible to determine a cause and effect relationship between the two in human studies. Owing to the limitations of human biopsy study, it was also difficult to determine in this study which genes were dysregulated by the augmented DNA methylation in failing hearts.

Conclusions

The present study revealed increased nuclear DNA methylation in cardiomyocytes, but not in non-cardiomyocytes, in biopsy specimens from the failing hearts of patients with DCM. The increased DNA methylation was predominantly localized in the heterochromatin and was significantly related to LV functional and structural parameters, although the link remains correlative. These findings are suggestive of the potential pathophysiological significance of DNA methylation in heart failure.

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Conflict of interest

None declared.

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