Desmin cytoskeleton remodeling in dilated cardiomyopathy

Elżbieta Czarnowska1, Agnieszka Pawlak2, Magdalena Brudek1, Lidia Ziółkowska3, Maciej Pronicki1, Wanda Kawalec3

1 Department of Pathology
2 Department of Cardiology
3 The Children’s Memorial Health Institute
Warsaw, Poland

Abstract

The aim of our study was evaluation of desmin distribution and expression in the myocardial tissue of patients with dilated cardiomyopathy (DCM) and assessment desmin remodeling with disease progression. We investigated endomyocardial tissue samples obtained during biopsy of the right ventricle. Samples obtained from 26 hearts with DCM were divided into three groups according to the duration of disorder symptoms and clinical features of heart failure as: (A) NYHA II and disease duration less than 3 months (5 patients, aged 8.62±3.17 year), (B) NYHA II and disease over 3 months (14 patients, aged 57.41±6.95 year), and (C) NYHA III and disease over 3 months (7 patients, aged 56.35±13.87 year). The left ventricle enlargement and ejection fraction were evaluated by echocardiography. Desmin distribution and expression was analyzed on paraffin sections immunohistochemically stained. Microscopical investigation showed that desmin cytoskeleton remodeling in cardiomyocytes is signified by increased desmin expression with normal pattern followed by its aggregation and then reduction or loss. Morphometric evaluation demonstrated that desmin aggregation increased with the disease duration and progress of heart failure, while desmin loss was characteristic feature of an early disorder stage and disorder duration longer than 3 months and presence of NYHA III. The tendency of the negative relationship between desmin aggregates contents and left ventricle ejection fraction was found. We conclude that desmin cytoskeleton remodeling occurs progressively from early DCM to heart failure and exhibits association with reduction of cardiac systolic function. Data suggest that increased desmin expression in DCM may be adaptive feature to counter abnormal elevation in wall stress when shows normal pattern and maladaptive when aggregates during increase cellular rigidity.

Key words: desmin, dilated cardiomyopathy, cytoskeleton, remodeling

Introduction

Dilated cardiomyopathy (DCM) independently of the etiology is characterized by left ventricle dilatation but normal wall thickness while systolic function is decreased. This disorder often leads to heart failure [10]. Experimental and clinical investigations indicate contribution of desmin, a protein of extrasarcomeric cytoskeleton to pathogenesis of DCM and cardiac dysfunction [12].

Desmin filaments form three-dimensional network around Z-disks of myofibrils keeping them in order and interlinking to each others, and connecting them to nuclei and sarcolemma, and positioning mitochondria [1]. Therefore this cytoskeletal network maintains cellular integrity and plays a role in force transmission and mechanochemical signaling.

Desmin mutations are associated with abnormal accumulation and cytoskeleton organization. While in myo-
cardiac disorders without genetic etiology, desmin expression varies and increases in cardiac hypertrophy and failing hearts [5,8], and decreases in end stage heart failure compared to controls [2]. Additionally, data suggest that desmin content in cardiomyocytes affects the long term prognosis in patients with heart failure [13].

Desmin cytoskeleton in DCM in relation to disorder stage and mechanisms which modify its remodeling remains to be elucidated. To ascertain DCM-associated desmin cytoskeleton remodeling we analyzed its patterns and optical density in relation to myocytes area and correlation to disorder duration and heart failure.

Materials and methods

Patient population

Twenty six patients enrolled in this study demonstrated left ventricular dysfunction as assessed by two-dimensional echocardiography, histopathological and clinical features of DCM and symptoms of heart failure (NYHA functional classes II and III). Patients with coronary heart disease, chronic alcoholism or heart failure of known origins, e.g. primary valvular disease were excluded from the study. All patients were treated with oral medications for heart failure (β-blockers, ACE inhibitors and diuretics).

In the studies there were three groups of patients according to disorder symptoms duration to biopsy and clinical features of heart failure:

(A) 5 patients (mean age 8,62±3,17 year) with NYHA II and disease duration less than 3 months;
(B) 14 patients (mean age 57,41±6,95 year) with NYHA II and disease duration over 3 months;
(C) 7 patients (mean age 56,35±13,87 year) NYHA III and disease duration over 3 months.

Clinical parameters of the ejection fraction and left ventricle enlargement of hearts belonging to the groups are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>EF [%]</th>
<th>LV enlargement [%]</th>
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<tbody>
<tr>
<td>Group A</td>
<td>44 ± 3,74</td>
<td>160,16 ± 22,83</td>
</tr>
<tr>
<td>Group B</td>
<td>35,85 ±0,14</td>
<td>114,28 ± 7,07</td>
</tr>
<tr>
<td>Group C</td>
<td>28,57 ± 9,44</td>
<td>118,28 ± 13,63</td>
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Immunohistochemistry

Tissue samples were fixed in 4% buffered formaline, embedded in paraffin, sectioned (thickness 5μm) and deparaffinized tissue sections were retrieved in Retrieval Solution (DakoCytomation) during 30 min and washed in Tris buffer pH 7,6. Sections were incubated with 0,03% hydrogen peroxide followed by 10% normal goat serum in phosphate buffered saline for 10 min to block endogenous peroxidase and non-specific binding respectively. Incubation with the primary desmin monoclonal mouse antibody (1:50, DakoCytomation) in Tris buffer pH 7,6 for 1 hour was performed at room temperature. After washes in Tris buffer incubation the secondary antibody goat anti-mouse conjugated with horseradish peroxidase (En Vision System HRP, DakoCytomation) for 30 min at room temperature was applied. To visualize the reaction AEC+Substrate Chromogen (DakoCytomation) was applied. Negative controls were obtained from procedure with omitted primary antibody.

Morphometry

Digital images of each sample were acquired under identical lighting conditions and optical setting (ColorView IIIu camera, Olympus) using x4 objective. Analysis were made using Cell® morphometric program (SIS Olympus, Germany). At first area myocytes – which was area of analytical interest, then the desmin area using automatic image threshold for increased and decreased or granular desmin expression was measured. The desmin area expressed by myocytes at certain level threshold was calculated as the % of cardiomyocyte area.

Statistical analysis

All date are expressed as mean value ± SD. Differences between means were compared using paired and unpaired Student’s t test. A value of P<0.05 was considered statistically significant. The relationships between continuous variables were evaluated by linear regression.

Results

The desmin in cardiomyocytes of all investigated myocardial samples formed network consisted of (i) thin fibrils exhibited a regular sarcomeric pattern along with intercalated disks (Fig.1A) or (ii) thick and high intensity stained fibrils exhibited a regular sarcomeric pattern along with perinuclear area and intensive staining in intercalated disks (Fig.1B) or (iii) massive perinuclear and beneath cell membrane clumps and tiny aggregates at Z disc (Fig.1C) or (iv) pale structures irregularly distributed (diffusive expression) which in some myocytes were lost (Fig.1D). We assumed that thin fibrils normally located exhibit normal desmin cytoskeleton. Thus increased desmin was expressed as thick fibrils and/or aggregates, while desmin loss by pale structures or lack of desmin. Quantity of cardiomyocytes presenting these three types of desmin network varied between the patients and investigated groups.

Morphometric analysis revealed the tendency to progressive increase of amount of cardiomyocytes with
intensive expression of the desmin as its total amount with intensively stained fibrils exhibited a regular sarcomeric pattern and massive aggregates when compared group A and B with NYHA II (Fig. 2). However, statistical significance differences for desmin increased expression evaluated as total of normal pattern and aggregates between these groups were not found. While the cell population with desmin aggregates showed the tendency to increase in relation to longer disease duration and heart failure (Fig. 3) and value of \( p \) was close to significance (\( p=0.06 \)) only when compared group A vs B. These findings suggest importance of the disorder duration for desmin cytoskeleton remodeling. The most numerous cardiomyocytes with reduced or lost desmin network were found in myocardial tissue of patients with an early disorder stage (disorder duration less than three months and NYHA II) and in patients with disorder duration longer than three months and NYHA III. Reduced expression of desmin network at early DCM phase was found nevertheless car-

![Fig. 1 Distribution of the desmin in myocardial tissue of the hearts with DCM: normal expression and distribution reflecting sarcomeric pattern (magnification x20) (A), normal distribution with increased expression expression (magnification x20) (B), increased expression in form of aggregates (magnification x40) (C), decreased or lost expression (magnification x40) (D)

![Fig. 2 Quantitative analysis of the cardiomyocytes area expressed normal and increased and decreased amount of desmin in DCM in respect to the disease lasting and symptoms of heart failure]

![Fig. 3 Quantitative analysis of the cardiomyocytes area expressed desmin in form aggregates in DCM in respect to the disease lasting and symptoms of heart failure]
Cardiomyocyte hypertrophy was not observed (cardiomyocyte diameter was from 15 to 30 μm).

Analysis of correlation between % of cardiomyocyte area with desmin expression and clinical parameters showed the tendency of negative correlation between desmin aggregation and left ventricle ejection fraction (Fig. 4).

**Fig. 4** Analysis of correlation between area of cardiomyocytes with increased expression of the desmin in form of aggregates and ejection fraction in DCM

### Discussion

In the present study we demonstrate abnormal pattern of the desmin in relation to the disorder lasting and heart failure and contractile dysfunction. Data suggest evolution of the desmin cytoskeleton remodeling from normal pattern to increased expression with normal pattern, followed by and increased expression in form of aggregates and then reduced expression and its relations with left ventricle ejection fraction.

**Desmin increased expression**

Increased expression of the desmin has been presented in human explanted hearts in association with the evolution and progression of heart failure [2, 5, 14]. Experimental data of immunohistochemical staining followed by western blot analysis solved that this pattern is not only staining intensity but also desmin accumulation [11]. It is not clear if this desmin increase is initiated by stress related to ventricle dilatation and precedes worsen ventricular function or an effect of distinct stimuli e.g. cardiomyocyte hypertrophy. It is suggested that increase of desmin arises with response to global alterations in stretch/stress to maintain cell compaction and to generate force. This hypothesis is supported by investigations of infarct effects in porcine model on the desmin expression showing increased desmin only in non-infarcted myocardial regions [15]. Additionally, none of the presented in literature studies have differentiated quantitatively the desmin increase in form of thick fibrils normally distributed from aggregates. Our morphological analysis revealed for the first time that quantity of aggregates increases systemically with disease progression. This form of desmin showed tendency of negative correlation with left ventricle ejection fraction. Thus, it seems that increased desmin expression with normal pattern is a compensative feature while desmin aggregates indicates on cytoskeleton degeneration. This concept is in agreement with observations of dilated cardiomyopathy related to desmin mutation in humans and animal model in which presence of aggregates leads to degenerative changes of cardiomyocytes [4, 6]. However, overexpression of mutant desmin leading to cardiomyopathy has not been related to cardiac function [16, 17]. It can not be excluded that appearance of the desmin aggregates is a key mark of an early degenerative cardiomyocyte changes.

**Desmin reduced expression**

Our study demonstrates that desmin reduction or loss is a characteristic feature of an early DCM phase (group A) and DCM with advanced heart failure (NYHA III, group C). However, increased fibrosis has been suggested in literature as the cause of desmin cytoskeleton abnormalities [11]. Our data of slight fibrosis (data not shown) reveled that desmin reduction in early phase DCM is not related to this mechanism. We also did not observe cardiomyocyte hypertrophy in early DCM. These hearts were characterized by significant enlargement of left ventricle (160% of normal heart). Therefore it can not be excluded that neither fibrosis nor hypertrophy but mechanical stress was cause of desmin cytoskeleton reduction. However, further studies are necessary for elucidating this hypothesis. Desmin reduction in our study co-existed with decreased left ventricle ejection fraction. This stays in agreement with data of others investigators [2, 13]. Pawlak et al provide data suggesting correlation of desmin cytoskeleton reduction with decreased heart function [13]. Interestingly, experimental models with absence of desmin leading to cardiomyopathy did not show its relation to cardiac function [3, 9, 18].

### Conclusions

Data suggest that remodeling of desmin cytoskeleton in DCM is initiated to stabilize the force generation and this process undergoes via amplification cellular network. While the process of transition DCM into heart failure is related to cytoskeleton disruption and reduction. Therefore the desmin increase might be a predictor of cell and heart function and remodeling process. Desmin might be novel target for therapeutic intervention since it has been shown that immunoabsorption therapy decreases desmin gene expression [7].

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References