Impairment of the myocardial desmin cytoskeleton and mitochondria alterations in heart diseases

Elżbieta Czarnowska¹, Magdalena Brudek¹, Agnieszka Pawlak², Agnieszka Sowińska¹, Lidia Ziółkowska¹, Anna Turska-Kmieć³, Wanda Kawalec³

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

³ Department of Cardiology
Central Hospital of Internal Affairs and Administration Ministry
Warsaw, Poland

Abstract
This study was aimed to determine the effect of desmin expression on changes of mitochondria number and morphology. Endomyocardial biopsy samples obtained from myocardial right ventricle for diagnostic reasons from 33 pts with determined finally heart disease as myocarditis (MC), dilated cardiomyopathy (DCM) with class NYHA II and III and from right atrium of 14 pts with myocardial septal defect with LV-RV systolic pressure gradient 21–64 mmHg (VSD I) and 67–97 mmHg (VSD II) were investigated by desmin immunohistochemistry and electron microscopy. The study revealed desmin expression presence: normal (type I), increased with normal distribution corresponding with Z lines arrangement and intercalated discs (type IIA) or increased as aggregates in perinuclear or perimembranous area in granular-like form (type IIB) in MC and DCM hearts. Desmin type IIA correlated with the presence of cardiomyocytes with normal ultrastructure but exhibiting increased number of varied morphology and size mitochondria in perinuclear area, while desmin type IIB with these containing remnants of myofilaments and numerous mitochondria non differentiated in shape and size. Among VSD hearts desmin type IIB and mitochondria non differentiated morphologically and often swollen were present in hearts VSD I. We discuss the desmin cytoskeleton expression type IIA and numerous varied mitochondria as compensative to some injury of contractile apparatus, while IIB and lost of mitochondria heterogeneity as morphological correlates with unfavorable disease progression.

Key words: desmin cytoskeleton, dilated cardiomyopathy, mitochondria, ventricular septal defect

Introduction
The desmin cytoskeleton in cardiomyocytes forms three-dimensional scaffold that maintains cellular organelles in normal arrangement and plays additionally mechanical and regulatory functions [18]. Changes of the cytoskeleton expressed as decreased or increased desmin immunostaining are known feature related to myocardial tissue injury. These has been shown in experimental studies [3, 14, 21, 23, 24] and human heart diseases [3, 5, 6, 8, 11, 16, 17, 20]. It has been suggested that desmin increased expression represents a stop-signal anchoring mitochondria in the specific cellular locations with highest energetic demands [2]. Mitochondria in these areas proliferate what is an adaptive feature. Than their morphological transformations, including giantism, swelling, change in shape, loss of cristae, decrease of matrix density occur [9]. Studies of mitochondria morphology and function have not yet been systematically carried on with references to stage of the disease or cellular remodeling and disease pathogenesis. From inves-
tigations of knockout animals is known that desmin null provides increase of mitochondria number and alteration of their morphology and decreased metabolic function [15]. This phenomenon also appears in overload hearts [25] and heart failure [7, 22].

Additionally, experimental data suggest that the mitochondrial alterations might be a secondary to a defect of the desmin organization [2]. What remodeling of the desmin cytoskeleton is pivotal for mitochondria alterations remains not clear. Therefore understanding the mechanism of desmin augmentation and its relation with mitochondria, could provide a scientific background for diagnosis of unfavorable disease stage.

In this study we verified what form of increased desmin expression correlate with mitochondria biogenesis to compensate energetic demands, and when augmentation of the desmin immunohistochemical staining together with mitochondria abnormal ultrastructural features may associate with unfavorable disease progression.

Materials and Methods

Myocardial tissue specimens

This study was approved by the ethics committee of The Children’s Memorial Health Institute.

Investigations were done on endomyocardial biopsy samples obtained due to diagnostic reasons from right ventricle of patients with determined finally heart disease as

(i) Myocarditis, class NYHA I and II (MC, 13 pts, age 10,99±4,89);

(ii) Dilated cardiomyopathy with no infiltration of inflammatory cells with clinical disease presentation about 3 month and class NYHA II (eDCM, 6 pts, age 9,95±3,87);

(iii) Dilated cardiomyopathy with no infiltration of inflammatory cells with disease presentation over 3 month, class NYHA II (iDCM, 7 pts, age 43,61±15,95);

(iv) Dilated cardiomyopathy with infiltration of inflammatory cells and disease presentation over 3 month, class NYHA III (iDCM, 7 pts, age 56,35±13,87);

and from patients undergoing transatrial surgical closure of ventricular septal defect with determined pressure gradient between LV-RV as

(v) 21–64 mmHg (VSD I, 7 pts, operation age 4–21 month);

(vi) 67–97 mmHg (VSD II, 7 pts, operation age 7–22 month);

Desmin expression

Deparaffinized tissue sections retrieved in Retrieval Solution (DakoCytomation) during 30 min were then incubated with 0.03% hydrogen peroxide and 10% normal goat serum to block the endogenous peroxidase and non-specific bindings, respectively. Incubation with the primary desmin monoclonal mouse antibody (1:50, DakoCytomation) for 1 hour followed by incubation with the goat anti-mouse secondary antibody conjugated with horseradish peroxidase (En Vision System HRP, DakoCytomation) for 30 min was performed at room temperature. Negative controls were obtained by omitting incubation with primary antibody in the procedure. Cytoskeleton expression was evaluated by morphometric manner presented below.

Ultrastructure

Two – three tissue samples obtained from one specimen were fixed by immersion in 2,5% glutaraldehyde in PBS and postfixed in osmium tetroxide, and then dehydrated in series of ethanol and acetone and proceeded into Epon 812 blocks. The typical appearance of cardiomycocytes and their abnormalities were analyzed under electron microscope (Jeol JEM 1011, Japan) and recorded by digital manner and then compared with tissue stained immunohistochemically with anti-desmin antibody.

Morphometry

Digital images of each sample were acquired under identical lightning conditions and optical setting (Olympus, Germany) using x4 objective. The images using automatic image threshold were digitally segmented for area with desmin expression as follow: normal (expression type I) or increased expression with normal distribution that corresponds with Z lines arrangement and intercalated discs (expression type IIA) or increased expression in form of granular-like aggregates (expression type IIB) or decreased expression and decay (expression type III) according to presentation previously shown [4].

The segmented cardiomycocytes with certain type of desmin expression were calculated automatically as the percentage of the total tissue area by using Cell morphometric program (Olympus, Germany). Morphometry measurements were made independently by two investigators.

Mitochondria size in cardiomycocytes was calculated from measurements of 20 objects per cells under transmisson electron microscope magnification x 20 000 using morphometric ITEM software (Jeol, Japan).

Statistical analyses

All data 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.

Results

Desmin expression

In diseased human myocardial tissue, desmin in significant number of cardiomycocytes was localized normally in areas of Z lines and intercalated discs, however with various intensity. The desmin normal intensity appeared as thin fibrils while increased intensity as thick fibrils. We distinguished the desmin normal intensity expression as type I, while increased intensity as type IIA. Desmin in various myocardial tissue areas irregularly distributed within the cardiomycocytes, exhibiting granular-like aggregates intensively stained was distinguished as type IIB. Desmin in various number of
myocytes formed pale structures or was not identified and these expressions were distinguished as type III. Representative images showing desmin localization in presented manner in diseased human myocardial tissue are shown in Fig. 1.

Percent of patients presenting the distinguished types of the desmin network varied between the investigated groups (Fig. 2). The highest index of desmin type IIA characterized the MC hearts and about 20% patients with DCM presentation about 3 month. While desmin type IIB was dominated in over 40% patients with DCM presentation over 3 month and class NYHA II (iDCM) and NYHA III (iDCM). Cardiomyocytes with desmin type IIB were often hypertrophic with irregular myofibrils network recognized in sections stained with hematoxylin and eosin. Desmin type III was expressed in 35%, 65%, 45% patients with myocarditis, DCM presentation about 3 month and over 3 month and NYHA II respectively. While among VSD hearts desmin type IIB or III was not observed in those with LV-RV systolic pressure gradient 24–64 mmHg.

Fig. 1 Representative images of desmin cytoskeleton expression: type IIA, (A), IIB (B) and III (C) in myocardial tissue of hearts with DCM and type IIA (D) and IIB (E) in VSD (D,E). Magnification x 50
The area of cardiomyocytes that express desmin type IIB increased in group DCM and class NYHA II significantly when disease persisted over 3 months in comparison with hearts presenting disease less period (Fig. 3).

General ultrastructure

Normal cardiomyocytes or with slightly increased diameter were significant part of cell’s population in MC and DCM with class NYHA II. These cardiomyocytes were characterized by increased number of oval mitochondria located mainly between myofibrils, seldom beneath of cell membrane (Fig. 4A). Hypertrophic cardiomyocytes were numerous in myocardial tissue of patients with DCM presentation over 3 months.
Investigations of various failing human hearts in the presented study indicated that desmin increased expression exhibits two stages represented by desmin type IIA and IIB. Similar observation has been presented in our earlier study of myocardial tissue from DCM hearts [4, 17]. In present the desmin type IIA predominantly occurred in myocardial tissue of patients with myocarditis and lower systolic pressure gradient between LV-RV in VSD I, while type IIB generally in those patients with DCM presentation over 3 month and class NYHA II and NYHA III and VSD II. These findings suggest that desmin type IIA is dominated feature in early stage of heart disease.

Cardiomyocytes with type IIA desmin remodeling exhibited normal ultrastructure with increased number of oval shaped or polymorphic and various size mitochondria, which accumulated in perinuclear area. These numerous mitochondria in perinuclear region might be a feature of their proliferation in area of high energy demand. Thus, desmin type IIA might express a signal for mitochondria proliferation in adaptive process.

Cardiomyocytes in myocardial tissue from hearts with VSD were normal diameter and possessed normal organized contractile apparatus but with numerous contraction bands and numerous mitochondria localized between myofibrils (Fig. 5).

Mitochondria

Increased number of mitochondria was a common feature of cardiomyocytes from all tissue samples (Fig. 4). They were located mainly between contractile apparatus and in perinuclear area and areas of myofibrils absence. These numerous mitochondria often formed clusters in cardiomyocytes from heart with MC and DCM presentation about 3 month and NYHA class II (Fig. 6A,B). Their shape varied from oval to polymorphic. Mitochondria characterized various size and shape particularly in cardiomyocytes with ultrastructure close to normal (Fig. 6C). Mitochondria in cardiomyocytes with atrophic myofibrils were mainly round shape and never formed clusters (Fig. 6D). Morphometric analysis revealed smaller size mitochondria in DCM persisted over 3 month in comparison to early stage of the disease (Fig. 7) Morphomorphic and differentiated in size and single giant mitochondria were present in VSD II (Fig. 6E) while in VSD I round, often swollen and loose crista (Fig. 6F).

Some of mitochondria in hearts with MC and VSD II were elongated.

Mitochondria matrix exhibited irregular density almost in all patients with MC and about 50% of eDCM and in over 80% patients with VSD I.

Discussion

Investigations of various failing human hearts in the presented study indicated that desmin increased expression exhibits two stages represented by desmin type IIA and IIB. Similar observation has been presented in our earlier study of myocardial tissue from DCM hearts [4, 17]. In present the desmin type IIA predominantly occurred in myocardial tissue of patients with myocarditis and lower systolic pressure gradient between LV-RV in VSD I, while type IIB generally in those patients with DCM presentation over 3 month and class NYHA II and NYHA III and VSD II. These findings suggest that desmin type IIA is dominated feature in early stage of heart disease.

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Cardiomyocytes in myocardial tissue with predominated desmin type IIB exhibited hypertrophy and ultrastructural changes consisted from rarefaction to absence of sarcomers and numerous mitochondria located in perinuclear regions and areas of myofibrils absence. Similar observations has been presented by Schaper et al. in end-stage dilated cardiomyopathy in which decreased metabolic function of mitochondria is known fact [20]. In experimental study was shown that accumulation of mitochondria in perinuclear region is characteristic feature of cardiomyocytes in which aggregates containing desmin were identified in the same area [1]. In our study these mitochondria were of various size and mainly round shape. It seems that mitochondria accumulated in perinuclear area and myofibrils absence might express degenerative cell remodeling related with the pres-
ence of desmin IIB. Thus round shape mitochondria might represent status of decreased metabolic function. Fact of decreased mitochondrial activity in myocardial tissue from hearts with idiopathic and ischemic dilated cardiomyopathy has been shown as secondary to the heart failure [10]. Additionally in heart failure some population of mitochondria become small what is in agreement with our data [19].

In literature has been also revealed that mitochondria intensive proliferation might be associated with their swelling and loss of structured cristae [2]. But in our study these was a characteristic feature of VSD with low systolic pressure gradient between LV-RV and increased desmin type IIA expression. Thus, it seems that mitochondria injury in this VSD patients were rather related with pathological conditions of pressure load and ischemia. This in agreement with presence of elongated mitochondria in MC and VSD. It is known, that elongated mitochondria may result of fission inability as effect of abnormal metabolic conditions [12].

**Fig. 6** Images of mitochondrial alterations in MC (A), DCM (B-D), and VSD (E-F) corresponding to desmin type IIA (C,F) and IIB (B,D, E) Magnification x 20000
Irregularity of mitochondrial matrix density in our study were observed mainly in MC and in patients with DCM presentation about 3 month and class NYHA II and over 3 month and NYHA III. Since in these hearts desmin type IIB and III dominated we can not exclude that desmin aggregates lost contact with mitochondria therefore do not affect their structure. Therefore, it seems that irregular matrix density is rather feature of cell failure. These is in agreement with recent data of Marin-Garcia and Goldenthal (2008) [13] and decreased mitochondrial metabolic function [for review see 9]. From earlier study of Sabbah et al. (1992) experimental canine heart failure model is known that in heart failure number mitochondria per unit volume increase, but they become smaller than conventional ones and have irregular matrix density [19].

Summarizing, it can not be excluded that increased expression of desmin cytoskeleton normal localized at sarcomers develops to compensate disorganized or partly lost contractile apparatus. Thus it is stabilize these cardiomyocytes survive. Fact that the desmin filaments may compensate sarcomers lost has been also suggested in the past by Schaper et al [20]. It seems that movement of mitochondria to perinuclear area and their increased proliferation in cardiomyocytes with normal desmin network but increased immunohistochemical expression meets energy demands. Therefore, further increase of desmin expression related with cytoskeleton injury and significant contractile apparatus injury and lost of mitochondria heterogeneity might be predictors of unfavorable disease progression.

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References