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RESEARCH ARTICLE

EXPRESSION OF DESMIN IN THE CONTEXT OF DESMIN GENE AND UBIQUITIN EXPRESSION IN PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY

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ABSTRACT

Background: Desmin as one of the major stress-bearing elements in the sarcomere and intercalated disk is an important determinant of cardiomyocyte function and long outcome. However, DES disturbances have been noticed in various heart diseases and can be modified by DES mRNA and ubiquitin proteasome system. The relation between desmin mRNA, desmin and ubiquitin in patients with idiopathic dilated cardiomyopathy (IDCM) has not been examined.

Aim: The evaluation of desmin and ubiquitin gene expression and ubiquitin expression in different types of desmin expression in cardiomyocytes in patients with IDCM.

Methods: Left ventricular endomyocardial biopsy was performed in 60 patients (85% males, mean age 46±14 years) with clinical symptoms of heart failure (HF) and left ventricular ejection fraction <45%. Expression and localization of desmin and ubiquitin were analysed in histological sections by immunohistochemical method using anti-desmin and anti-ubiquitin (DAKO) antibodies. Desmin mRNA expression and localization was determined by FISH methods. Western-blott (W-b) was performed to quantify analysis of desmin and ubiquitin. Patients were assigned to four types according to the desmin expression in cardiomyocytes: type I, 9 patients with normal desmin expression; type IIA, 23 patients with high desmin expression in physiological localisation, type IIB, 20 patients with high desmin expression and desmin aggregates; group III, 8 patients with low desmin expression in cardiomyocytes.

Results: In group I, even and weak expression of ubiquitin in the cytosol and low expression of desmin mRNA in cytosol and nuclei of cardiomyocytes was observed. The expression of ubiquitin and desmin mRNA increased along with the progression of desmin cytoskeleton remodeling (type IIA and IIB). Desmin mRNA and ubiquitin were weakly expressed or not present in myocardium of patients from desmin type III. The desmin mRNA, desmin and ubiquitin expression in cardiomyocyte was associated with gradual changes in cardiomyocyte structure (increase in cardiomyocytes hypertrophy and fibrosis) as well as HF progress (higher NYHA class, increase in the level of N-terminal pro-brain natriuretic protein and left ventricular end diastolic diameter and decrease in left ventricular ejection fraction).

Conclusions: The ubiquitin and the mRNA of desmin can modify the level of desmin expression. Increase in expression of ubiquitin and desmin mRNA might be a feature associated with protection an unfavorable cell remodeling, which reduces the adverse effects of cytoskeleton damage in the early stage of HF. It seems that the lack of ubiquitin and low desmin mRNA expression can be a marker of the end stage HF.

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INTRODUCTION

Idiopathic dilated cardiomyopathy (IDCM) is characterized by progressive increased hemodynamic load and ventricular dilatation, which initiates in early stage of disease compensatory mechanisms (Osadchii *et al*., 2007). In the end stage of IDCM the inability to maintain defense mechanisms is observed. Desmin is one of the most important cytoskeletal, intermediate filament proteins which plays an essential structural, mechanical and regulatory role in cardiac integrity. It is a dynamic structure which is constantly destroyed, renewed or newly constructed (Paulin *et al*., 2004; Bruce Alberts *et al*., 2008). Its alterations including changes in

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mass and remodeling of cytoskeleton are known cellular compensatory mechanisms (Balasubramanian *et al*., 2006; Tolstonog *et al*., 2002; Lazarides *et al*., 1980). Increased expression and aggregation of desmin as well as decreased expression have been noticed in various heart diseases and have been proved as crucial factors for the prognosis of patients with IDCM (Goldfarb *et al*., 1998; Li *et al*., 1999). Therefore, both identifying and understanding mechanisms which regulate desmin expression and localisation in different stages of heart failure (HF) are substantial to indicate the possibility of their modification. Desmin missence mutations in humans have previously been reported and resulted in restrictive cardiomyopathy or IDCM (Goldfarb *et al*., 1998; Li *et al*., 1999). The animal models with mutations of the desmin gene develop cardiomyopathy with the degeneration of cardiomyocytes, fibrosis and ultrastructural defects in the cardiac muscle which reduce animals' life spam and make them less tolerant of exercise (Paulin *et al*., 2004; Thornell *et al*., 1997; Wang *et al*., 2001). On the other hand, the crucial role of ubiquitin proteasom system in homeostatic protein turnover in the myocardium for proper cardiac function is widely accepted (Wang and Robbins, 2014). The ubiquitin, a small modifier protein, tags proteins that are either abnormal or no longer needed for degradation via the proteasome (Mukhopadhyay and Riezman, 2007; Schnell and Hicke, 2003). The conjugation of the ubiquitin with proteins in the ubiquitination process plays an essential role in nearly all aspects of cell function including compensatory response. It has reported the importance of hyperubiquitination of proteins in IDCM (Weekes *et al*., 2003). Cohen *et al*. proved the possibility of desmin ubiquitination (Cohen *et al*., 2012). In our study we have tested the hypothesis that different levels of desmin gene expression and changes in ubiquitin expression may coexist with different patterns of desmin expression and localisation in cardiomyoctes in consecutive stages of HF. For this purpose, we studied the correlation between the expression of desmin, desmin mRNA and ubiquitin and both cardiomyocyte ultrastructural changes and clinical parameters in patients with IDCM.

MATERIALS AND METHODS

Study population and clinical assessment

Baseline examinations were performed between January 2010 and January 2012. Patients presenting with IDCM were enrolled prospectively and consecutively in the study. Inclusion criteria were age >18 years; clinical diagnosis of IDCM; left ventricular ejection fraction (LVEF)<45% assessed by echocardiography; and clinical stability. Exclusion criteria were significant coronary artery disease on coronary angiography (defined as the presence of any stenotic lesion with >50% reduction in lumen diameter). Clinical, laboratory and echocardiographic assesment was performed on admission. The serum N-terminal-pro-brain natiuretic peptide (NT-pro-BNP,pg/mL) concentration was maesured on the Cobas e411 System (Roche Diagnostic GmbH) device with the use of immunoassey based on electroimmunofluorescence. The transthoracic echocardiography examination was carried out using a commercial diagnostic ultrasound system equipped with a 3.5-Hz transducer (iE 33; Philips Medical System, Best, the Netherlands). All measurements, left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD) and LVEF were performed according to the guidelines of the European Society of Echocardiography (Evangelista *et al*., 2008) by an experienced cardiologist blinded to the patients' clinical history. LVEF was assessed using Simpson's method to estimate volume. Left ventricular endomyocardial biopsy (EMB) was performed using a 7F bioptome (Cordis; Johnson & Johnson Co, New Brunswick, NJ, USA). The procedure was carried out under continuous ECG monitoring. Six tissue samples were collected from each patient. Hearts from donors with normal left ventricular function (that were not used for transplantation) served as controls. The local ethics committee of the Central Clinical Hospital of the Ministry of Internal Affairs, Warsaw, Poland, approved the study protocol. Informed written consent was obtained from every study participant. The study was conducted in accordance with the Declaration of Helsinki. The characteristics of the entire study population is presented in Table 1.

Table 1. Clinical characteristics of the study groups

Variables	Study population (n=60)
Gender, male (%)	51 (85)
Age, years	46±14
BMI, kg/m^2	26±5
NYHA class, n (%)	
Class I,	16 (27)
Class II,	27 (45)
Class III,	12 (20)
Class IV,	4 (7)
LVEF, %	34±14
LVEDD, mm	64±14
NT-pro-BNP, pg/mL	1420 (331-2705)
Comorbidities, n/(%)	
Hypertension	20 (33)
Diabetes melitus	4 (6)
Hypercholesterolemia	6 (9)
Medication, n (%)	
ACE - I	53 (83)
ARB	6 (9)
B-blockers	52 (81)
Aldosteron antagonists	46 (72)
Diuretics	32 (50)

BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker. Data are presented as means ± standard deviations, medians (with lower and upper quartiles) or number (with percentages) where appropriate.

Histopathology

Two tissue samples from each patient were evaluated histologically with haematoxylin and eosin (H&E), desmin and ubiquitin stains. Samples were incubated first with desmin monoclonal mouse anti-human antibody (1:50; DAKO, Glostrup, Denmark) or ubiquitin polyclonal rabbit anti-human antibody (1:150; DAKO, Glostrup, Denmark) and secondly with a horseradish peroxidase–conjugated goat anti-mouse antibody (En Vision System HRP; DAKO). Negative controls

were obtained by omitting incubation with the primary anti-desmin antibody or anti-ubiquitin antibody. Total desmin or ubiquitin levels in the biopsy samples were analysed using Western blotting. Cardiac tissue protein was harvested using a cell lysis buffer (150 mmol/L sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and 50 mmol/L Tris, pH8.0). Total protein (20µg) was separated by 10% SDSPAGE. Western blotting was performed using desmin monoclonal mouse anti-human antibody (1 : 500; DAKO), the ubiquitin polyclonal rabbit anti-human antibody (1:1000; DAKO) and the rabbit monoclonal anti-smooth muscle actin antibody (1:100; Abcam, Cambridge, MA,USA). The signal was revealed using Alkaline Phosphatase (AP) Conjugate Substrate kit (Bio-Rad, Hercules, CA,USA) after hybridization with goat anti-mouse IgG (H+L) (Bio-Rad) AP-conjugated secondary antibodies. The membranes were scanned using VersaDocTM Imaging System (Bio-Rad). Band densities were analysed using Quantity One software (Bio-Rad). Two independent pathologists evaluated two sections prepared from each biopsy sample. Image analysis was performed using the same lighting conditions and optical settings for each sample. Immunohistochemical staining of desmin revealed 4 types: i) type I, normal, gentle staining of desmin at Z-lines and intercalated disks, giving a regular cross-section pattern; ii) type IIA, increased, intensive staining of desmin at Z-lines and intercalated disks, giving regular cross-striation pattern; iii) type IIB, increased, intensive staining of desmin with an irregular cross-striation pattern and/or with the presence of aggregates in perinuclear areas and, occasionally, in intermyofibrillar spaces; and iv) type III, decreased or lack of desmin staining. The full classification of desmin expression in cardiomyocytes was previously published (Pawlak *et al*., 2012). Immunohistochemical staining of ubiquitin revealed 4 types of ubiquitin expression: i) type A, gentle, unevenly scattered expression of ubiquitin in the cytoplasm of cardiomyocytes; ii) type B, increased, evenly scattered expression of ubiquitin in the cytoplasm of cardiomiocytes; iii) type C, increased, unevenly scattered expression of ubiquitin in the cytoplasm of cardiomyocytes and its increased, evenly scattered expression in nucleus; and iv) type D, lack/weak expression of ubiquitin (Figure 1). Each type of desmin or ubiquitin expression was measured and automatically calculated as the percentage of the total defined tissue section. The dominant immunohistochemical pattern of desmin or ubiquitin staining in tissue sections determined the type of cytoskeletal desmin or ubiquitin expression. Cardiomyocyte and nucleus hypertrophy and tissue fibrosis were assessed in tissue sections stained with H&E and TM. Images of whole sections were taken under the same lightning conditions and under the same optical settings using an x4 objective with the use of the morphometric image analysis Cellp software (Olympus, Hamburg, Germany).

Fluorescent in situ hybridization (FISH)

Tissue samples were postfixed in 4% paraformaldehyde (PFA) (Sigma-Aldrich) in PBS for 24 h, cryoprotected with 30% sucrose (Sigma-Aldrich), and snap-frozen on dry ice. In situ hybridization was performed on 30-µm thick sections on poly-prep slides (Sigma-Aldrich). The coding sequence of human desmin mRNA in pOTB7 plasmid was purchased from imaGenes (nr. IRAVp969H0179D). Sense and antisense fluorescein labeled riboprobes were generated from plasmids with SP6 or T7 polymerase sites. Specimens were fixed in 4% PFA, washed in PBS and incubated in acetylation buffer (0.1 M triethanolamine, 0.25% HCl, 0.25% acetic anhydride). Next, sections were prehybridized for 3 hours in prehybridization solution (Sigma-Aldrich) followed by overnight hybridization at 70°C in hybridization solution (Sigma-Aldrich) containing desmin sense or antisense probes. Afterwards, sections were washed in 0.2x SSC, with first two washes carried out for an hour in 70°C, then five consecutive washes for 30 min in RT. After then, TNB blocking solution (TSA Plus System, Perkin Elmer) was applied for an hour. Tissue samples were incubated with antifluorescein-POD (Roche Applied Science) 1:200 and desmin monoclonal mouse anti-human antibody (1:50;DAKO,Glostrup, Denmark) overnight in 4°C, washed with PBST and incubated with secondary antibody, 1:1000 Alexa 488-conjugated anti-mouse IgG (Invitrogen). Hybridization signal was amplified with Cy3 TSA Plus System (Perkin Elmer). Analysis revealed different patterns of desmin mRNA expression in cardiomyocytes i) type 1, even but weak expression in nuclei and in cytosol, ii) type 2, uneven but intensive expression, more fequent localisation in nucleus than in cytosol, iii) type 3, intensive expression in cytosol and the same or weaker expression in nucleus, iv) type 4, very low expression in cytosol and lack in nucleus.

Statistical analyses

Continuous variables were expressed as mean ± standard deviation (SD), continuous variables with a skewed distribution were expressed as median with lower and upper quartiles and categorical variables were expressed as percentage. Intergroup differences were tested using Student's t-test where as ANOVA was used for multiple group comparisons, and frequencies were compared using chi-squared test. Pearson's correlation coefficients were calculated between NYHA class, NT-pro-BNP levels, LVEDD, LVEF for each type of desmin expression. All tests were two sided with a significance level of $p < 0.05$. A commercial statistical package (SPSS 13.0; IBM, Armonk, NY, USA) was used for all statistical analyses.

RESULTS

Desmin and ubiquitin expression in histopathology

In our study population, the type I of the desmin expression in cardiac tissue was observed in 9(15%) patients, type IIA in 23(38%), type IIB in 20(33%) and type III in 8(13%) patients. The type A of the ubiquitin expression was presented in 9(15%) patients, type B in 24(40%), type C in 19(32%) and type D in 8(13%) patients. In the cardiac tissue samples, type A of the ubiquitin expression coexisted with type I of desmin expression in 89% and with type IIA in 11%. Type B of ubiquitin expression was mainly observed in type IIA of desmin expression (84%) and occasionally in type I (4%), type IIB (8%) and type III (4%). Type C of ubiquitin expression was found in tissue samples from patients with type IIB of desmin expression in 95% and with type IIA in 5%. The decrease or lack of the ubiquitin expression in cardiomyocytes

was accompanied by low or lack desmin expression (type III) in cardiomyocytes in 90% (Table 2, Figure 1). In patients with normal expression of desmin immunohistochemistry revealed weak or lack of the ubiquitin expression and its low quantity. Ubiquitin expression increased along with the progression of desmin cytoskeleton remodeling, mainly in the cytoplasm of cardiomyocytes with compensatively increased desmin network (desmin type IIA), and in the cytoplasm and in the nuclei of cardiomyocytes with the degeneration of the desmin network (desmin type IIB). Ubiquitin was not found or weekly expressed in biopsy samples collected from patients with decreased or the lack of desmin expression. The highest sensitivity and the lowest specificity was observed for ubiquitin type C and type IIB desmin expression, 87,5% and 38,5%, respectively.

Table 2. Presence of different types of desmin expression in biopsy samples from patients with different types of ubiquitin expression

		UBIQUITIN n (%)			
		Type A 9(15)	Type B 24(40)	Type C 19(32)	Type D 8(13)
DESMIN (%)	Type I 9(15)	8(89)	1(11)	0	0
	Type IIA 23(38)	1(4)	20(87)	1(4)	1(4)
	Type IIB 20(33)	0	2(10)	18(90)	0
	Type III 8(13)	0	1(13)	0	7(87)

Content of desmin and ubiquitin in cardiomyocytes in Western Blot

Differences in the desmin and ubiquitin expression were confirmed by Western blotting. When compared to type I, the content of desmin was increased by 63% and 23% in types IIA and IIB, respectively, and decreased by 18% in type III of desmin expression (Figure 1). The content of ubiquitin in biopsy samples from patients with IDCM was increased by 91% and 101% in type B and C and decreased by 76% in type D to type A, respectively.

Desmin mRNA expression in FISH

The desmin mRNA was expressed evenly but weakly in the cardiomyocytes (nucleus and cytosol) in patients with type I of desmin expression. Increase in desmin content in the cardiomyocytes (type IIA) was accompanied by an increase in desmin mRNA expression mainly in the nuclei. In type IIB of desmin expression with further excessive desmin expression, the progression of the disarrangement of desmin (very elegantly presented in the fluorescent immunohistochemistry– Figure 2) and the accumulation of protein aggregates in the cardiomyocytes, the increased expression of mRNA level was seen not only in the nuclei, but also in the cytoplasm of the cardiomyocytes. The lack of desmin mRNA in the nuclei and its very low distribution in the cytoplasm of the cardiomyocytes was observed in type III of desmin expression (Table 3, Figure 2). In IDCM hearts, a double labeling fluorescent immunohisochemistry was applied to localize the immunoreactivity for desmin and desmin mRNA. This metod clearly revealed the colocalization of the changes in desmin and desmin mRNA.

Cardiomyocytes diameter and fibrosis in desmin and ubiquitin types

The mean cardiomyocyte diameters were 19.5, 26.1, 28.6 and 29.4μm in types A, B, C and D ubiquitin expression, respectively. In biopsy samples, mean fibrosis were 13.2, 16.4, 26.8, 26.9 percentage of area slides in types A, B, C, D ubiquitin expression, respectively. The mean cardiomyocyte diameters were lower in all types of desmin expression in comparison with the corresponding types of ubiquitin expression and they were 18.0, 25.8, 29.1, 30.9 in types I, IIA, IIB, III respectively. The analysis of fibrosis in different types of desmin expression also showed smaller area percentage of fibrosis than in the similar types of ubiquitin expression and they were 12.9, 19.3, 24.1, 29.1 in types I, IIA, IIB, III respectively.

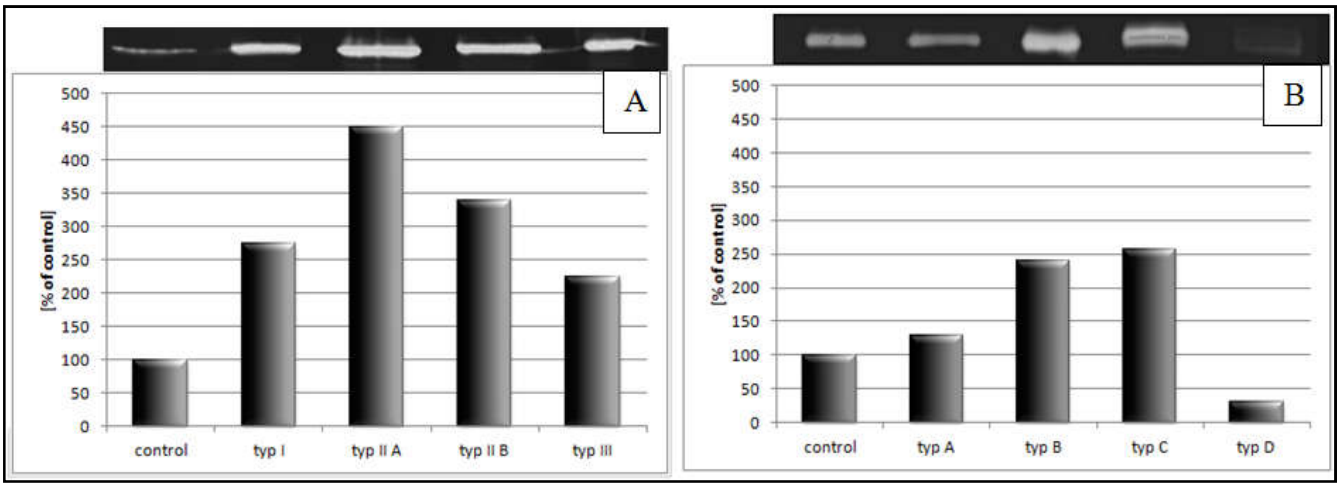

Figure 1. Western blot analysis showing control (heart donors) and different types of desmin (A) and ubiquitin (B) expression (patients with idiopathic dilated cardiomyopathy)

Table 3. Types of expression and localisation of desmin, ubiquitin and desmin mRNA in cardiomyocytes of patients with IDCM

		TYPES OF EXPRESSION and LOCALIZATION			
	Type	I	IIA	IIB	III
	Localisation	Cytoplasm	Cytoplasm	Cytoplasm	Cytoplasm
DESMIN	Expression	Gentle	Intensive	Intensive, Aggregates	Weak/lack
	Type	A	B	C	D
	Localisation	Cytoplasm	Cytoplasm	Cytoplasm and nuclei	Cytoplasm
UBIQUITIN	Expression	Gentle	Intensive	Intensive	Weak/lack
	Type	1	2	3	4
	Localisation	Cytoplasm and nuclei	Cytoplasm and nuclei	Cytoplasm and nuclei	Cytoplasm
DESMIN mRNA	Expression	Even, weak	Uneven, and intensive, mainly in nuclei	Even, Intensive	Weak/lack

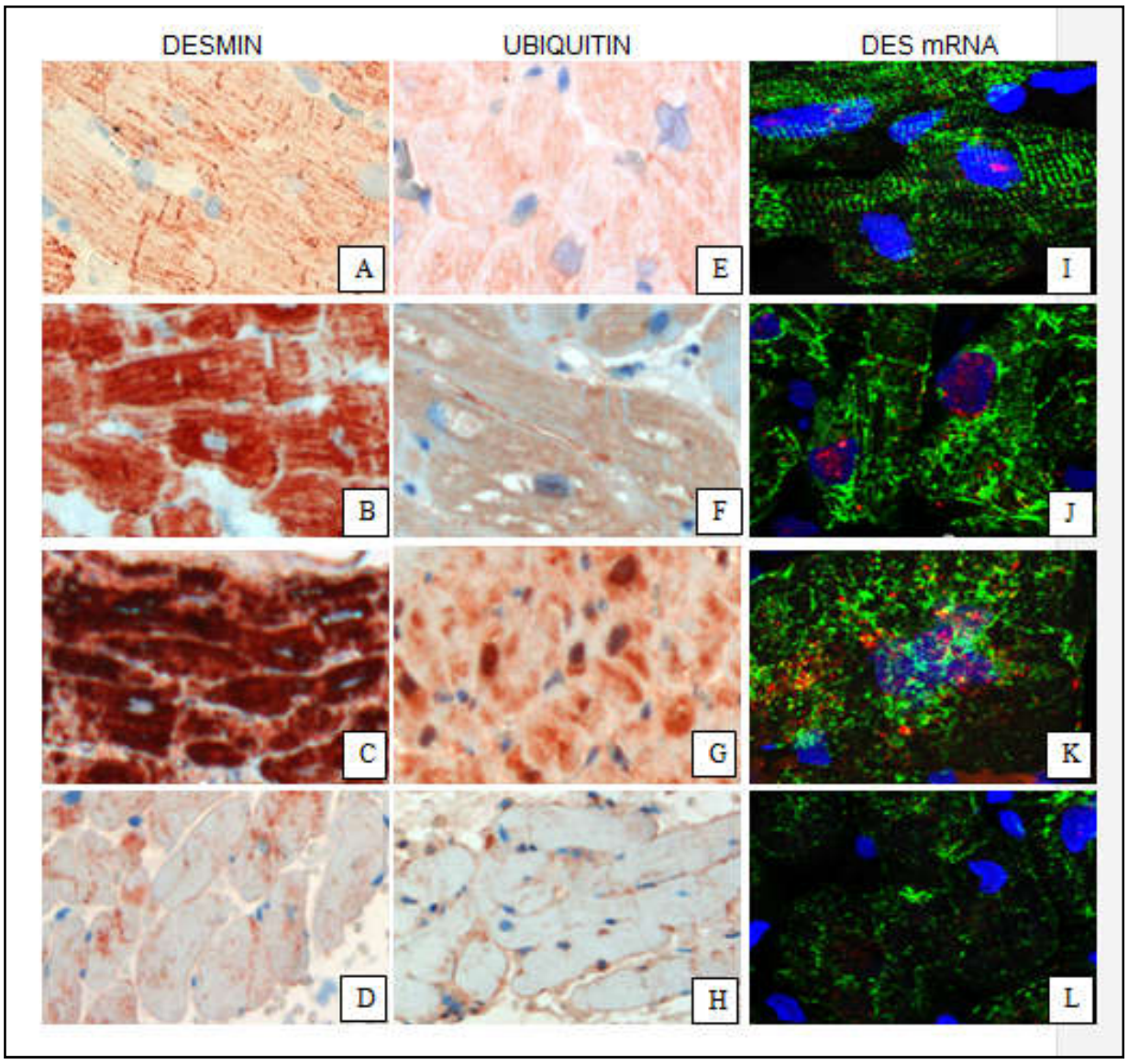

Figure 2. Immunohistochemical analysis of desmin, ubiquitin and desmin mRNA in the heart of patients with IDCM. Immunohistostainings with desmin antibodies (type I - A, type IIA-B, type IIB-C and type III- D), ubiquitin antibodies (type A-E, type B- F, type C -G and type D-H) evaluated in the light microscopy. Hybridization signal observed in tissue sections (I, J, K and L). Nuclei in each tissue section were stained with DAPI (I, J, K and L). Overlay shows the localization of desmin (green colour) and desmin mRNA (red colour). In patients with IDCM desmin mRNA proteins were localized in the cytoplasm and in the nuclei of cardiomyoctes. Desmin mRNA showed even, weak expression both in the nuclei and in the cytoplasm (type 1- I), intensive expression, more pronounced in the nuclei than in the cyoplasm (type B-J), intensive expression in the cytoplasm and the same or weaker expression in the nuclei (type 3 - K) and the lack of desmin mRNA in the nuclei and very weak distribution in the cytoplasm (type 4 - L)

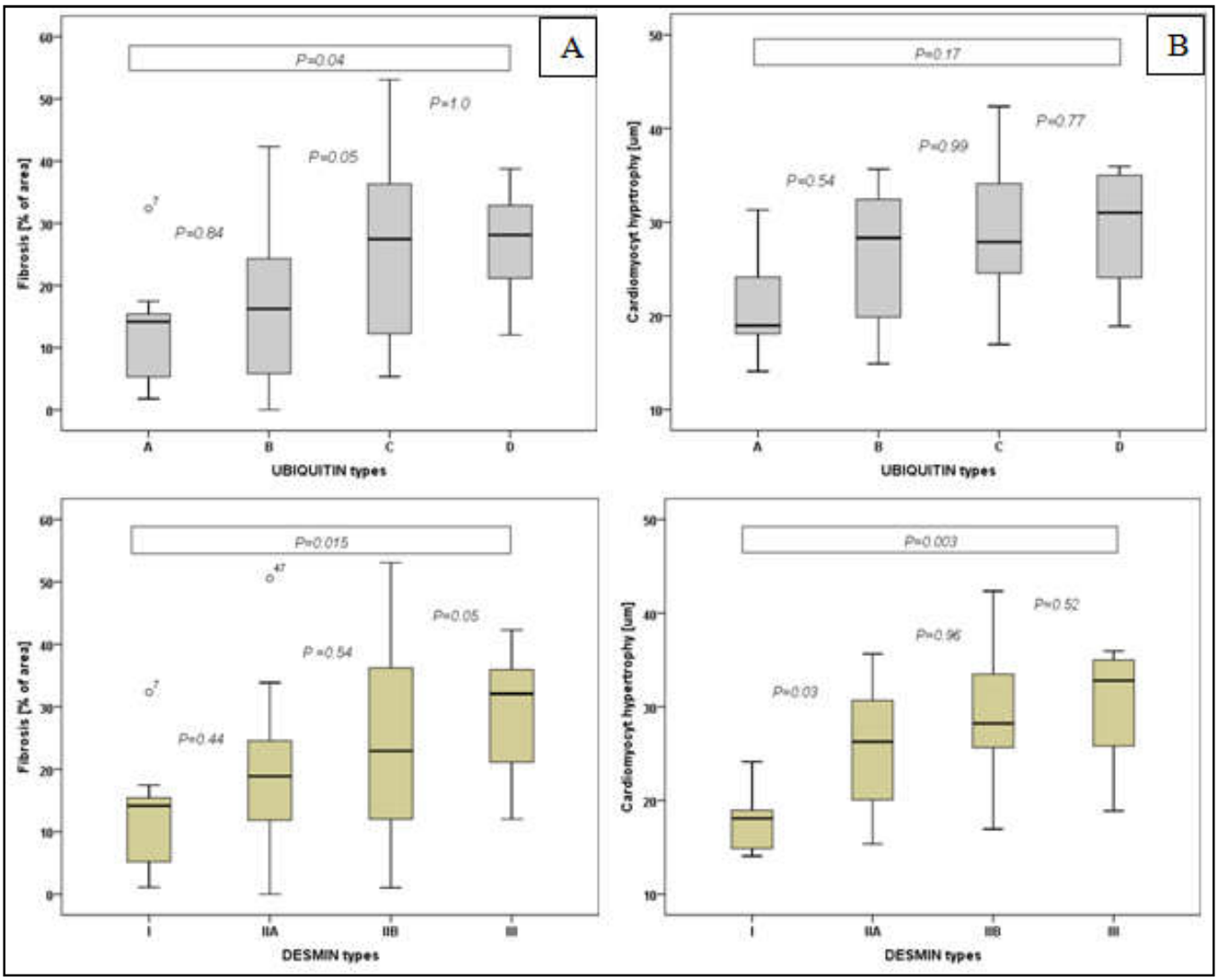

Figure 3. Box plots showing percentage of brous tissue area (A for each type of ubiquitin expression) and cardiomyocyte hypertrophy (B for each type of ubiquitin expression). Data show mean ± SD

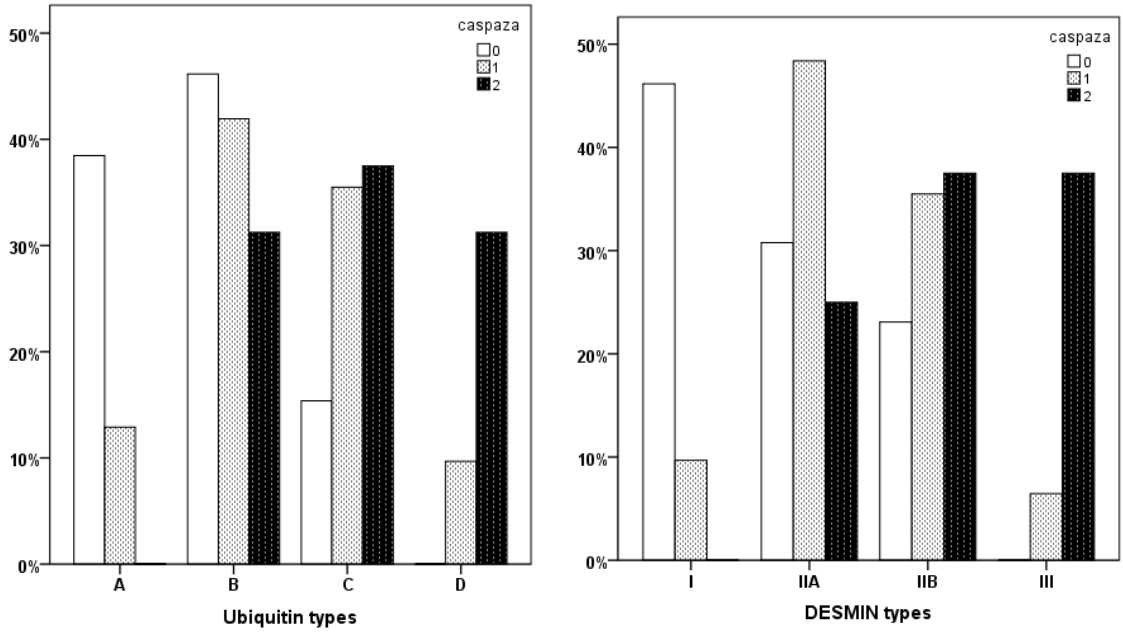

Figure 4. Caspase expression in patients with different types of ubiquitin and desmin expression (0-lack of caspase expression in slides, 1- caspase expression in single cardiomyocytes in slides, 2 –caspase expression in numerous cardiomyocytes in slides)

Table 4. Echocardiografic parameters, NT-pro-BNP level and NYHA class in different types of desmin and ubiquitin expression. Data show mean ± SD

	NYHA	NT-pro-BNP	LVEF	LVEDD
Desmin				
Type I	1.4±0.7	336±280	47.9±13	58.9±8
Type IIA	1.9±0.7	1556±657	36.2±16	61.3±10
Type IIB	2.0±0.8	1923±1221	27.8±8	66.2±8
Type III	2.2±0.6	1725±573	27.5±7	67.3±6
P	0.2	0.2	0.001	0.2
R	0.3	0.2	-0.5	0.2
Ubiquitin				
Type A	1.6±0.9	772±453	44.8±10	58.3±6
Type B	1.8±0.6	1331±765	37.6±11	61.8±8
Type C	2.0±0.8	2155±1232	27.2±7	66.2±5
Type D	2.1±0.7	1562±631	27.5±7	67.3±5
P	0.6	0.03	0.002	0.1
R	0.2	0.2	-0.4	0.3

P – p-value beetwen desmin and NYHA class, NT-pro-BNP level, LVEF and LVEDD and ubiquitin and NYHA class, NT-pro-BNP level, LVEF and LVEDD; R – correlation coefficient.

Caspase expression in different types of desmin and ubiquitin expression

Both in consecutive types of desmin and ubiquitin expression increasing caspase expression was observed. In patients with ubiquitin type A and desmin type I, caspase was absent or present in single cardiomyocytes. In type B and C of the ubiquitin expression and IIA and IIB of desmin expression three types of caspase expression were seen in cardiac tissue (lack of caspase expression, caspase expression in single cardiomyocytes, caspase expression in numerous cardiomyocytes). In patients with type D and III caspase was expressed either in single or in numerous cardiomyocytes. In types D and III, cardiomyocytes without caspase were not found (Figure 4).

Clinical presentation in the different type of desmin and ubiquitin expression

The analysis of clinical parameters such as NYHA class, biochemical such as NT-pro BNP and echocardiografic such as LVEF and LVEDD revealed similar values of mentioned above parameters in patients assigned to the corresponding types of desmin and ubiquitin expression. The significant correlation was observed only between the type of desmin and LVEF and betwen the ubiquitn expression and NT-pro-BNP level and LVEF (Table 4).

DISCUSSION

Despite extensive literature on the essential role of desmin in IDCM, desmin turnover in cardiomyocytes in consecutive stages of HF is not clear. We analysed the relationship between changes in desmin gene expression, desmin cytoskeleton remodeling and ubiquitin expression in human hearts failing. In the present study imunohistochemistry revealed for the first time: i) different patterns of protectively enhanced expression of desmin mRNA and ubiquitin in cardiomyocyte, which seems to be a mechanism compensating the increased desmin cytoskeleton turnover (early phase) and the progression in disorganisation of desmin network (late phase) and ii) the lack or decrease in desmin mRNA and ubiquitin expression in the end-stage of IDCM, with progressive decreasing of desmin expression which may indicate decompensated phase of the human heart. The evaluation of the elements that play a role in the desmin tunover, in the context of cell structure and clinical presentation, is an attempt to explain complex process of HF development.

Ubiquitin patterns of expression

Changes in ubiquitin expression have previously been described in experimental models as well as in patients with IDCM (21,23). To our knowledge, this is the first study systematically assessing the distribution and expression of ubiquitin classified as types A, B, C and D in relation to the desmin and desmin mRNA expression in patients with IDCM. The ubiquitin expression followed by ubiquitination plays a protective and adaptive role by the modification of growth and transcription factors, preventing proteins aggregation, which are harmful to the cardiomyocyte, inhibiting of apoptosis by ubiquitination of caspases (Osadchii *et al*., 2007; Wang and Robbins, 2014; Wang and Terpstra, 2013; Otsuka *et al*., 2010; Johnson *et al*., 2004). All above, in consequence are in favour of remodeling and hypertrophic growth of cardiomyocytes. Weekes *et al*. reported that the ubiquitination of a number of proteins was enhanced in IDCM hearts (Weekes *et al*., 2003). Moreover, the recently discovered ligase TRIM32 indicates the possibility of post-translational modification of desmin by ubiquitin (Cohen *et al*., 2012; Otsuka *et al*., 2010). The colocalisation and simultaneous increase in the expression of desmin and ubiquitin observed in our study may indicate desmin ubiquitination. Equal increase in the ubiquitin expression (type B) could be a mechanism compensating increase in the expression of desmin (type IIA) in the early phase of the compensation of IDCM. Interestingly that, increased expression of ubiquitin exists not only in cells with protein aggregates, as it was shown ealier, but also in cardiomyocytes without protein aggregates. Significant growth of ubiquitin content was also confirmed in this phase by Western blotting. It has been reported that, in responce to cellular stress, liable proteins are modified and that most modified proteins easily misfold, aggregate and form intracellular inclussions where ubiquitin accumulates (Johnston

et al., 1998; Lowe *et al*., 1988). Thus, the ubiquitin positive granular structures observed in cardiomyocytes are considered to be aggregates of modified proteins, which are ubiquitinylated. Cohen *et al*. suggested that the enhanced phosforylation of desmin filaments during fasting increases their ubiquitylation by Trim 32 and depolymerization and degradation (Cohen *et al*., 2012). In consequence, desmin disaarangement and accumulation of aggregates can be observed and moreover the breakdown of Z-bands and thin filaments. Furthermore, with progress of IDCM, the further modification of desmin can promote increase in and enhance TRIM 32 ligase-modified degradation of the desmin cytoskeleton, which appears to facilitate the breakdown of Z-bands and thin filaments. It can explain changes in type of desmin expression (type IIB), ubiquitin expression (type C) as well as in contractile apparatus and finaly may lead to cardiomyocytes degradation (Cohen *et al*., 2012). In consequence, desmin disaarangement and accumulation of aggregates, which can overwhelm the proteasomal degradative system cause apoptosis, autophagic myocyte loss and HF progress (Mukhopadhyay and Riezman, 2007; Wang and Terpstra, 2013). Therefore, it is not suprising that in this phase (late phase of compensation of IDCM) we have noticed increase in caspase expression in numerous cardiomyocyte in slide. In decompensated phase of HF, decrease in or lack of desmin mRNA, desmin or ubiquitin expression was observed in our patients. It remains difficult to interprete, as there is no data concerning the result of mRNA desmin, desmin and ubiquitin expression in patients with IDCM. The exhaustion of compensatory mechanisms in cardiomyocytes could be the reason of low mRNA and protein expression. The significant decrease in number of mitochondria, their shape and localisation observed in ealier studies may confirm our suspicion (Pawlak *et al*., 2012; Mackiewicz *et al*., 2012).

Desmin mRNA patterns of expression

We showed that desmin is significantly increased not only at the protein level but also at the mRNA level as it was described previously (Pawlak *et al*., 2013; Kallwellis-Opara *et al*., 2007; Heling *et al*., 2000). It may be speculated that in early compensated phase of IDCM (desmin mRNA-type 2, desmin – type IIA, ubiquitin –type B), the increased desmin expression is not only regulated by ubiquitin as it was indicated previously, but also by desmin mRNA (Kallwellis-Opara *et al*., 2007). However, the ubiquitination of transcription factors is not dominative process leading to increase in desmin mRNA since the expression of ubiquitin in nuclei has been almost not observed in our patients. It is interesting that, in late, compensated phase the presence of desmin mRNA was observed not only in nuclei, but also in cytoplasm, in the most, in the cardiomyocytes with the intensive disarrangement of desmin (type IIB) and ubiquitin expression in the nuclei and in the cytoplasm (type C) at the same time. Investigation regarding mRNA and ubiquitin expression performed in experimental model indicate that the increase in expression of mRNA may be the effect of increased ubiquitination of some transcription factors (Otsuka *et al*., 2010). The excessive scattered expression of ubiquitin in nuclei in this phase presumably confirms this hypothesis (Muratani and Tansey, 2003). Moreover, the increase in expression of desmin mRNA in the cytoplasm in type IIB, in our present study, seems to be also associated with the ubiquitination process as a consequence of increasing activation of family E3 ligases. Durairaj *et al*. proved that the HECT ubiquitin ligases as well as the cullin RING ligase CSFMdm30 are connected with the transport of mRNA from the nucleus to the cytoplasm (Durairaj *et al*., 2009). Based on our data, we suspect that increase in desmin mRNA expression may be considered as an important feature of early and late phase of compensatory mechanism. Additionaly, the possibility of the regulation of desmin mRNA expression in heart by beta-blockers, immunoadsorption and subsequent immunoglobulin substitution creates a new therapeutic option for patients with IDCM (Kallwellis-Opara *et al*., 2007).

Desmin mRNA and ubiquitin expression versus cell structure and clinical presentation

Our study revealed that the progressive increase in desmin mRNA, desmin and ubiquitin expression in cardiomyocyte was associated with gradual changes in cardiomyocyte structure and HF progress. However, in end-stage HF, although many cardiomyocytes exhibit a decrease in or lack of desmin mRNA, desmin and ubiquitin expression the further progress in cariomyocytes hypertrophy and fibrosis were noticed. The significant cardiomyocytes hypertrophy and irrelevant increase in fibrosis in early compensative phase of IDCM as well as significant increase of fibrosis only in late compensative phase are consistent with the elegant study of Mackiewicz at al., who suggested that extracellular matrix remodeling seems to be secondary to the primary changes in cardiomyocytes (Mackiewicz *et al*., 2012) (Figure 2). Moreover, the disarrangement of desmin rather than fibrosis per se, may play an important role in the development of IDCM (Mackiewicz *et al*., 2012). It is possible that both paracrine effects as well as cell to cell contacts participate in crosstalk between myocytes and fibroblast (Kakka and Lee, 2010). The higher value of myocyte diameter and fibrosis displied in ubiquitin type than in comparable desmin type may indicate that ubiquitin could be a more precise marker of myocardium remodeling. Rubin *et al*. reported that the ubiquitynilation of growth fators is one of the most important signalling cascade controlling myocyte hypertrophy (Rubin *et al*., 2005). Our study shows that changes in the desmin mRNA, ubiquitin and desmin expression were corelated with clinical, echocardiographic and biochemical parameters. The most favourable LVEF abd LVEDD measurements, the lowest NT-pro-BP levels and NYHA functional class characterised patients with: type 1-desmin mRNA, type A – ubiquitin and type I desmin expression. In patients with the increase expression of all analysed proteins and mRNA in early and late compensation phases we found unfavourable and gradually exacerbate values of LVEF, LVEDD, NT-pro-BNP and NYHA class. It seems that these adverse values

Are related to the accumulation of proteins can contribute to cell death, aggregation of abnormal cytotoxic proteins, which play a role in cardiomyocytes degeneration (Otsuka *et al*., 2010) The decopmensate phase of IDCM was associated with the highest NYHA class, the highest NT-pro-BNP, the biggest LVEDD and the lowest LVEF values. The corellation between

desmin mRNA, desmin and ubiquitin expression and these four parameters emphasizes the importance of these proteins in IDCM pathology. Changes in the desmin cytoskeleton regarding the structural features and clinical parameters were described in the prevoius study (Pawlak *et al*., 2012)

Conclusion

The ubiquitin and the mRNA of desmin can modify level of desmin expression. Increase in expression of ubiquitin and desmin mRNA might be a feature associated with protection an unfavorable cell remodeling, that reduces the adverse effects of cytoskeleton damage in the early stage of HF. It seems that lack of ubiquitin and low desmin mRNA expression can be a marker of the end stage HF. Therefore, the current findings of concomitant expression of demin mRNA, desmin and ubiquitin may present the chain of processes in which the desmin expression is regulated.

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