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Original article

Preserved cardiomyocyte function and altered desmin pattern in transgenic mouse model of dilated cardiomyopathy

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ABSTRACT

Taking advantage of the unique model of slowly developing dilated cardiomyopathy in mice with cardiomyocyte-specific transgenic overexpression of activated Gαq protein (Tgαq*44 mice) we analyzed the contribution of the cardiomyocyte malfunction, fibrosis and cytoskeleton remodeling to the development of heart failure in this model. Left ventricular (LV) *in vivo* function, myocardial fibrosis, cytoskeletal proteins expression and distribution, Ca^{2+} handling and contractile function of isolated cardiomyocytes were evaluated at the stages of the early, compensated, and late, decompensated heart failure in 4-, 12- and 14-month-old Tgαq*44 mice, respectively, and compared to age-matched wild-type FVB mice. In the 4-month-old Tgαq*44 mice significant myocardial fibrosis, moderate myocyte hypertrophy and increased expression of regularly arranged and homogenously distributed desmin accompanied by increased phosphorylation of desmin chaperone protein, αB-crystallin, were found. Cardiomyocyte shortening, Ca^{2+} handling and LV function were not altered. At 12 and 14 months of age, Tgαq*44 mice displayed progressive deterioration of the LV function. The contractile performance of isolated myocytes was still preserved, and the amplitude of Ca^{2+} transients was even increased probably due to impairment of Na^{+}/Ca^{2+} exchanger function, while fibrosis was more extensive than in younger mice. Moreover, substantial disarrangement of desmin distribution accompanied by decreasing phosphorylation of αB-crystallin appeared. In Tgαq*44 mice disarrangement of desmin, at least partly related to inadequate phosphorylation of αB-crystallin seems to be importantly involved in the progressive deterioration of contractile heart function.

1. Introduction

Multiple pathological changes have been reported in failing hearts at the organ, tissue and cellular levels. Hypertrophy and/or chamber dilatation along with the impairment of hemodynamic parameters (e.g. ejection fraction, end-diastolic and end-systolic left ventricular pressure) are key changes at the organ level. At the tissue level, myocyte hypertrophy and remodeling of the extracellular matrix (ECM) dominate. Changes in ECM entail increased fibrosis, collagen isoforms (I and III) shifts and cleavage of parts of the collagen net that are directly connected with cardiomyocytes (*endomisium* and *perimisium)* [1,2]. Their destruction combined with disturbances in costameric proteins results in disruption of bidirectional force transmission between myocytes and ECM [3]. At the cellular level changes in structure of cytoskeleton were reported. Among the wide range of cytoskeletal proteins desmin plays pivotal role [4,5]. It connects Z discs of contractile apparatus with costameric proteins in the sarcolemma enabling transmission of contractile force to the ECM. Moreover, desmin enables mechanical connection between the individual myocytes in desmosomes. Desmin also provides connection between sarcolemma and nuclear as well as mitochondrial membranes, thus influencing localization and function of these organelles. The role of desmin in heart remodeling is not clear. It has been recently shown that remodeling of desmin might be a more accurate and sensitive measure of cardiac dysfunction in heart failure than myocardial fibrosis [6]. In explanted human hearts failing because of dilated cardiomyopathy Heling et al. [7] found significant increase in desmin

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expression at mRNA and protein level and its intracellular disorganization. However, Pawlak et al. [8] have shown recently decreased, normal as well as increased desmin content in biopsies from failing human hearts.

In myocardial tissue from patients with dilated cardiomyopathy desmin distribution was irregular, with its lack in some cytoplasmic regions and increased density in perinucler and intermyofibrilar spaces [9,10].

Desmin remodeling may result from changes in function of its chaperone protein αB-crystallin, small heat shock protein, abundantly expressed in the myocardium [5]. Mutations of αB-crystallin gene lead to abnormal aggregation of cytoskeletal proteins including desmin [11]. Overexpression of αB-crystallin, in turn, results in less tissue damage in mice hearts subjected to ischemia and reperfusion [12], as well as in protection from overload-induced hypertrophy [13]. The disarrangement of cytoskeleton may lead to deterioration of heart contractility by impairment of individual cardiomyocyte contractility and disruption of force transmission between cardiomyocytes and ECM. Cardiomyocyte contractile function may be also impaired in the failing hearts by the changes in Ca^{2+} handling proteins. The most often described are the decrease in expression and activity of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA), increased diastolic Ca^{2+} leak through Ca^{2+} release channels of the SR (ryanodine receptors — RyRs) and various changes of Na^{+}/Ca^{2+} exchanger (NCX) function (increased as well as decreased activity) [14,15]. Decreased SERCA function, especially when combined with increased NCX function and Ca^{2+} leak results in decreased SR content and failure of contractile function. Besides affecting contractility, disturbances in Ca^{2+} handling are strongly arrhythmogenic [16].

Despite extensive literature of the biology of failing hearts, the temporal and causal relationship between changes in myocardial ECM, cytoskeleton remodeling, and myocyte performance is not clear. In humans, myocardial tissue is mostly obtained at the end-stage heart failure at which virtually all changes have already developed. In animal models with relatively rapid development of heart failure after experimental injury (e.g. rapid-pacing, myocardial infarction, aortic banding) it is difficult to establish the sequence of events leading to end-stage heart failure.

In contrast, Tgαq*44 mice with targeted overexpression of HA-tagged, constitutively activated Gαq (HAαq*) protein in cardiomyocytes display a relatively slow progression of the heart failure: they are symptom-free for approximately 12 months and then develop severe dilated cardiomyopathy with both diastolic and systolic dysfunction mimicking the phenotype of human dilated cardiomyopathy within next couple of months [17–20]. Interestingly, increased hypertrophic marker gene (ANP, BNP and MHC-β) expression is evident already at 4 months [17,21]. Thus, this model provides a unique possibility to examine the sequence of events from the initial overactivation of Gq-PLCβ signaling to the progression of heart failure and premature death by 14–19 months of age.

Moreover, in Tgαq*44 mice overexpression of activated Gαq protein mimics the effects of stimulation of Gq-coupled receptors by angiotensin II, endotelin-1 or by noradrenaline, the neuroendocrine factors, which play a key role in the development of heart failure in the experimental models as well as in humans.

In Tgαq*44 mice we previously characterized alterations in PKC- and PKA-dependent cardiomyocyte signaling [17,20], mitochondrial dysfunction [18], development of oxidative stress and coronary endothelial dysfunction [19], late alterations in myofilaments protein expression and active and passive force generation in permeabilized cardiomyocytes [20]. However, the relative contribution of cardiomyocyte malfunction, ECM and cytoskeleton remodeling to the development of dilated cardiomyopathy in this model is not known. Thus, the aim of the present study was to explore the relative contribution of alterations in cardiomyocyte function and Ca^{2+} handling and changes in cytoskeletal proteins content and arrangement as well as myocardial fibrosis, to the development of LV dysfunction in dilated cardiomyopathy in Tgαq*44 mice.

2. Material and methods

2.1. Animals

Tgαq*44 mice were generated as described previously [17]. Homozygous Tgαq*44 mice and wild-type mice (FVB) were bred in the Animal Laboratory of the Institute of Experimental and Clinical Medicine of the Polish Academy of Sciences in Warsaw. Genomic DNA was extracted from tail biopsies and genotyping was performed using polymerase chain reaction (PCR) according to Mende et al. [22]. Mice were housed in pathogen free conditions, fed a standard laboratory diet and given water *ad libitum*. All measurements were performed in female Tgαq*44 mice and age-matched wild-type FVB mice (wt) at the age of 4, 12 and 14 months. Additionally, fibrosis was evaluated also at the age of 2, 6 and 8 months.

All animal procedures conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996), and the experimental procedures used in the present study were approved by the local Ethical Committee on Animal Experiments.

2.2. Assessment of cardiac function in vivo by MRI

Cardiac function was analyzed *in vivo* as described previously [18,21]. Mice were anesthetized with 2% isoflurane via a nose cone. Animals were positioned in the probehead supine position and their temperature was stabilized with the flow of warm air at 35 °C. Magnetic resonance imaging (MRI) was performed with an ECG triggered gradient echo (cine-like flow compensated FLASH) sequence. For assessment of LV volumes and dynamics cine in the midventricular short-axis slice was performed with the following imaging parameters: echo time (TE) 2.5 ms, acquisition matrix 128×128, view field (FOV) 30 mm^2, slice thickness 1.5 mm, number of scans per heart 8. Flip angle was set to achieve the best contrast between myocardium and blood pool and repetition time (TR) was adjusted to the R-R period so that at least 20 frames per cardiac cycle were acquired in the midventricular short-axis projection.

LV slice areas were evaluated using automatically or semi-automatically delineated images of endocardium in all the acquired frames. For this task the Aphelion v.3.2 (ADCIS-AAI) package for image analysis was used. Slice areas were measured in all acquired frames and plotted against the acquisition time resulting in an area-time curve. Subsequently, the curve was filtered using running average filter with step 2. The end-systolic (ESA) and end-diastolic (EDA) areas were measured and fractional area change (FAC) (indicative of ejection fraction, EF) was calculated as (EDA–ESA)/EDA. From the area–time curves the ejection rate (ER) and filling rate (FR) were obtained as described previously [21].

2.3. Measurements of cardiac and myocyte hypertrophy and myocardial fibrosis

Animals were anesthetized (ketamine HCl 150 mg/kg and xylazine 10 mg/kg body weight, intraperitoneal injection), hearts were excised and weighed with and without atria. Overall enlargement of the heart was assessed by total ventricular/body weight, while lung edema was evaluated by measuring wet/dry lung weight.

For light microscopy, whole heart was fixed in 10% buffered formalin (pH 7.4) and paraffin-embedded. Tissue samples were cut into thick sections. Areas of fibrosis were quantified in seven fields of three ventricular sections (5 μm) stained with Trichrom Masson with microscope (Olympus) at 40× magnification using Cell^{P} (SIS, Olympus) software that corresponded to a tissue area of 0.98 mm^2/