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Research Article

Sarcomeric lesions and remodeling proximal to intercalated disks in overload-induced cardiac hypertrophy

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ABSTRACT

Pressure overload induces cardiac remodeling involving both the contractile machinery and intercalated disks (IDs). Filamin C (FlnC) and Xin actin-binding repeat-containing proteins (XIRPs) are multi-adapters localizing in IDs of higher vertebrates. Knockout of the gene encoding Xin *(Xirp1)* in mice leads to a mild cardiac phenotype with ID mislocalization. In order to amplify this phenotype, we performed transverse aortic constriction (TAC) on control and *Xirp1*-deficient mice. TAC induced similar left ventricular hypertrophy in both genotypes, suggesting that the lack of Xin does not lead to higher susceptibility to cardiac overload. However, in both genotypes, FlnC appeared in "streaming" localizations across multiple sarcomeres proximal to the IDs, suggesting a remodeling response. Furthermore, FlnC-positive areas of remodeling, reminiscent of sarcomeric lesions previously described for skeletal muscles (but so far unreported in the heart), were also observed. These adaptations reflect a similarly strong effect of the pressure induced by TAC in both genotypes. However, 2 weeks post-operation TAC-treated knockout hearts had reduced levels of connexin43 and slightly increased incidents of ventricular tachycardia compared to their wild-type (WT) counterparts. Our findings highlight the FlnC-positive sarcomeric lesions and ID-proximal streaming as general remodeling responses in cardiac overload-induced hypertrophy.
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1. Introduction

Cardiac hypertrophy is characterized by a growth of cardiomyocytes in width, length, or both. Such growth can be physiological, as in the case of remodeling in response to exercise, or pathological, due to abnormal overload or disease – both types mediated by distinct mechanisms [1]. In either case, cells have to grow by the addition of new sarcomeres. It was proposed that new sarcomeres are added at or near the intercalated disc (ID) region [2,3], the area of the cell where myofibrils terminate and connect to the membrane and the neighboring cell via specialized structures called adherens junctions. Indeed, a recent study in which pathological hypertrophy was surgically induced in rabbits showed that sarcomeres are progressively inserted from the ID in a stepwise process [4]. Analysis of two mouse dilated cardiomyopathy (DCM) models which display pathological hypertrophy suggested that it is this addition process that is misregulated in DCM [5]. This further highlighted the role of the ID in the regulation of hypertrophy and sarcomere number, in both health and disease.

In skeletal muscles, the structure corresponding to the ID is the myotendinous junction (MTJ), and even though experimental evidence is lacking, it is plausible that new sarcomeres could also be added from there during hypertrophy. However, eccentric exercise in skeletal muscles (which is hypertrophy-inducing [6,7]) mainly gives rise to sarcomeric "lesions", which are areas of remodeling that can be found anywhere within a muscle fiber [8–12]. These lesions, which may span as little as one sarcomere or extend over large numbers of them, always border on Z-disks, are positive for both Xin actin-binding repeat-containing proteins (Xin and XIRP2) and FlnC [12–14]. These structures may simply represent areas of repair after myofibrillar damage, but it has also been proposed that they are areas of addition of new sarcomeres necessary for cell growth [12,15,16]. Similar sarcomeric lesions

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have not been documented in either healthy or pathological hypertrophic hearts so far.

XIRPs are expressed in striated muscles and localize predominantly in IDs of cardiomyocytes and MTJs of skeletal muscles [17–19]. They are characterized by so-called Xin repeats, conserved 16 amino acid motifs binding to actin filaments [20–22]. The chicken genome seems to contain only one *XIRP* gene [23] and inactivation of *XIRP1* in chicken embryos resulted in looping defects, abnormal heart beating and pulmonary edema [24], indicating that *XIRP1* plays a key role in cardiac morphogenesis. By contrast, the zebrafish genome contains three *XIRP* genes [23,25], and in mammals, XIRPs are encoded by two genes [20], *XIRP1* and *XIRP2* (in the mouse *Xirp1* and *Xirp2*). The latter were considered to be candidates for cardiac diseases due to their co-expression with human cardiomyopathy-associated genes [26]. Mammalian *XIRP1* gives rise to three Xin isoforms: XinA, XinB and XinC [18]. Mice deficient for all isoforms ($Xirp1^{tm1Dofr}/Xirp1^{tm1Dofr}$ or $XinABC^{-/-}$ mice, henceforth referred to as $Xin^{-/-}$) had ID abnormalities, such as localization of ID-like structures to the lateral part of cardiomyocytes and increased numbers of gap junctions, explaining an elevated conduction velocity [27]. Despite this, there was no electrophysiological evidence for increased atrial or ventricular vulnerability or propensity toward development of cardiac hypertrophy.

Altogether, $Xin^{-/-}$ mice only show a mild cardiac phenotype. Given this situation, we hypothesized that upon cardiac stress the effects of Xin-deficiency would be amplified and translate not only into further morphological alterations but also a cardiac electrophysiological response. An established technique for the experimental induction of cardiac stress and hypertrophy in the mouse is transverse aortic constriction (TAC) [28,29]. The surgically inserted banding narrows the transverse aorta, forcing the left ventricle to pump more strongly resulting in its hypertrophy. We, therefore, applied TAC in $Xin^{-/-}$ mice to induce pressure-induced cardiac hypertrophy as a surrogate for cardiac stress. In our analysis, we only found small differences between the TAC-treated WT and $Xin^{-/-}$ mice. However, both genotypes showed FlnC-positive areas of remodeling proximal to the IDs, and a prevalence of sarcomeric lesions - a hallmark of eccentric exercise remodeling - previously unreported in the heart.

2. Materials and methods

2.1. Experimental groups

This study was performed on 14-week old female $Xin^{-/-}$ mice, generation of which was based on the mixed 129/B6 background as previously described [27]. For comparison, WT mixed 129/B6 mice were used and randomized to undergo either TAC or sham operation procedure (SOP). Consequently, four groups were formed: $Xin^{-/-}$ TAC and SOP along with WT TAC and SOP. Numbers of animals for cardiac measurements: WT SOP, n=13; WT TAC, n=9; $Xin^{-/-}$ SOP, n=12; $Xin^{-/-}$ TAC, n=13; for electrophysiology experiments, numbers are stated in the results tables; for Western blot analyses, numbers are stated in appropriate section. Animal procedures complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (Publication No. 85-23, revised 1996). Treatment protocols were approved by the "State office for protection of the environment, nature, and consumers" of the district government of North Rhine-Westphalia in Recklinghausen, Germany.

2.2. TAC

TAC was performed to evoke pressure overload-induced heart failure. Mice were anesthetized with isoflurane (2 vol%, Forene®, Abbott GmbH, Wiesbaden, Germany), intubated and mechanical ventilation was established (MiniVent 845, Hugo Sachs Elektronik, March-Hugstetten, Germany). To confer a standardized degree of aortic constriction a 27-gauge needle was used as a placeholder to pass a suture underneath the aortic [30]. SOP controls underwent the same procedure but were not ligated. Before surgery, mice were given 0.1 mg/kg of buprenorphine subcutaneously for analgesia. 3 or 14 days after surgery mice were euthanized. Immediately prior to euthanization, they were assigned at random to undergo either hemodynamic or electrophysiological evaluation. Subsequently, hearts were explanted and prepared for further analysis.

2.3. Biometric measurements

All animals were weighed immediately before and two weeks after surgery. Two weeks after TAC or SOP, cardiac biometric parameters were determined. The lung and heart were excised (the latter divided into left and right ventricle) weighed and prepared for further analysis. Tibia length was measured. The left ventricles were snap frozen in liquid nitrogen and stored at −80 °C.

2.4. Hemodynamic measurements

Hemodynamic evaluation was conducted 14 days after TAC or SOP using a 1.4-Fr Millar (Millar Instruments; Houston Texas) catheter as described previously [31] after steady state analgesia with isoflurane (1 vol%, Forene®, Abbott GmbH, Wiesbaden, Germany) had been achieved. In brief, the right carotid artery was dissected under a microscope and the catheter was introduced into the aorta and subsequently advanced into the left ventricle. At each level, hemodynamic parameters were recorded by a transducer connected to a computerized data acquisition system (PowerLab, ADInstruments, Grand Junction, Colorado). The LabChart 6 data analysis software (ADInstruments Ltd) was used to calculate hemodynamic parameters, including heart rate, peripheral systolic and diastolic pressure, left ventricular systolic and diastolic pressure, the rate of left ventricle (LV) pressure rise in early systole (dP/dt_{max}) and the slope of the LV diastolic pressure decrement (dP/dt_{min}).

2.5. Surface electrocardiography (ECG) and electrophysiological investigation

Electrophysiological characterization and analysis were performed as described previously [32–34]. Mice were anesthetized with exhalative isoflurane (2 vol%, Forene®). A 6-lead surface ECG was monitored continuously and analyzed under stable conditions for three minutes. For in vivo electrophysiological investigation, the jugular vein was dissected and a 2.0-Fr octapolar mouse electrophysiological catheter (eight 0.5 mm circular electrodes; electrode-pair spacing 0.5 mm (Ciber Mouse, NuMed Inc., NY, USA)) was placed into the right heart on the atrial and ventricular level. Intracardiac electrograms and transvenous atrial and ventricular stimulation maneuvers were recorded as previously described [33,34]. Fixed rate and extrastimulus pacing, sinus node recovery time, Wenckebach periodicity, atrial refractory periods and atrioventricular nodal refractory periods (ARP, AVNRP) were evaluated. The ventricular refractory period was evaluated by ventricular extrastimulus pacing. (S1S1: 120, 110 and 100 ms followed by up to three extra beats).

Atrial fibrillation (AF) was defined as rapid and fragmented atrial electrograms with irregular AV-nodal conduction for ≥ 1 s. AF was induced by atrial burst stimulation. Ventricular tachycardia (VT) was defined as four or more ventricular ectopic ventricular beats. Ventricular vulnerability was tested by ventricular burst stimulation for 1 s. Number of inducible AF and VT episodes and the probability of arrhythmia induction were analyzed.

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