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Atomic force and electron microscopic-based study of sarcolemmal surface of living cardiomyocytes unveils unexpected mitochondrial shift in heart failure

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ABSTRACT

Loss of T-tubules (TT), sarcolemmal invaginations of cardiomyocytes (CMs), was recently identified as a general heart failure (HF) hallmark. However, whether TT per se or the overall sarcolemma is altered during HF process is still unknown. In this study, we directly examined sarcolemmal surface topography and physical properties using Atomic Force Microscopy (AFM) in living CMs from healthy and failing mice hearts. We confirmed the presence of highly organized crests and hollows along myofilaments in isolated healthy CMs. Sarcolemma topography was tightly correlated with elasticity, with crests stiffer than hollows and related to the presence of few packed subsarcolemmal mitochondria (SSM) as evidenced by electron microscopy. Three days after myocardial infarction (MI), CMs already exhibit an overall sarcolemma disorganization with general loss of crests topography thus becoming smooth and correlating with a decreased elasticity while interfibrillar mitochondria (IFM), myofilaments alignment and TT network were unaltered. End-stage post-ischemic condition (15 days post-MI) exacerbates overall sarcolemma disorganization with, in addition to general loss of crest/hollow periodicity, a significant increase of cell surface stiffness. Strikingly, electron microscopy revealed the total depletion of SSM while some IFM heaps could be visualized beneath the membrane. Accordingly, mitochondrial Ca^{2+} studies showed a heterogeneous pattern between SSM and IFM in healthy CMs which disappeared in HF. In vitro, formamide-induced sarcolemmal stress on healthy CMs phenocopied post-ischemic kinetics abnormalities and revealed initial SSM death and crest/hollow disorganization followed by IFM later disarray which moved toward the cell surface and structured heaps correlating with TT loss. This study demonstrates that the loss of crest/hollow organization of CM surface in HF occurs early and precedes disruption of the TT network. It also highlights a general stiffness increased of the CM surface most likely related to atypical IFM heaps while SSM died during HF process. Overall, these results indicate that initial sarcolemmal stress leading to SSM death could underlie subsequent TT disarray and HF setting.

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1. Introduction

Most cardiovascular diseases are characterized by important changes in the extracellular matrix (ECM) components correlating with an increase of tissue stiffness contributing to the alteration of heart function [1–5]. However, recent works indicate that, besides ECM, individual cells within the organ tissue also undergo large morphological and elasticity modifications under pathological conditions [6–8] sometimes much earlier than functional dysfunctions.

To face a pathological myocardial stress, cardiomyocytes (CM), the contractile cell entity of the heart, change their morphology [6] but also their signaling [9] and electro-mechanical properties [10,11] to stabilize their contractile function. However, for still unknown reasons, they switch over time to a maladaptation state progressively leading to CM death and thus to the onset of heart failure (HF). Recently, remodeling and loss of T-tubules (TT) were identified as a general hallmark in a broad spectrum of late stage HF models [10,12–14] and was further characterized as an early and progressive event occurring during HF development [13]. This HF phenotype was also correlated with the modifications of the β-adrenergic cAMP signaling compartmentalization taking place at the CM surface [15]. More largely, alterations in the overall CM ionic functional surface were associated with failing CM phenotype [12,15]. Collectively, these data highlight the potential involvement of architectural alterations of the CM sarcolemmal membrane in the setting of HF. However, these CM alterations were generally observed at a final HF stage with most probably some alterations appearing consequently to other ones. Thus, because TT are longitudinal invaginations of the sarcolemma occurring at the Z-line, whether TT network per se or more specific sarcolemmal alterations subsequently leading to TT disarray occur during the onset of HF is still largely ignored.

In this study, we combined Atomic Force Microscopy (AFM) and electron microscopy to directly characterize both surface topography and nanomechanical properties of sarcolemma of living CMs from healthy or failing mice hearts.

2. Materials and methods

An expanded methods section is available in the Supplementary information.

2.1. Atomic Force Microscopy (AFM) and Force Measurement

Atomic Force Microscopy experiments were conducted in CM culture medium, at 37 °C under 5% CO_2 flow, using the perfusing cell and the heater system from the Bioscope Catalyst (Bruker, Santa Barbara, CA, USA). For all imaging modes (tapping, contact, Force volume), we used the same bare MLCT AFM probes which present a pyramidal tip made of Si_3N_4, with a curvature radius of 35°, manufactured by Bruker. AFM height images were recorded both in contact or tapping mode. In contact mode the tip is scanned over the surface while applying a constant force. A feedback loop adjusts the tip height in order to keep the force constant allowing deduction of height images. In tapping mode, the cantilever is oscillated at its resonance frequency and the tip is scanned over the surface while keeping a reduction of the cantilever amplitude constant. Once again, a feedback loop is in charge of keeping the amplitude decrease constant in order to deduce height images. The cantilever spring constants were systematically measured using the thermal tune method [16] and has been found to range from 10 to 30 pN/nm. The maximal force applied to the cell has been limited to 2 nN in order to preserve the membrane integrity. The force (F) versus displacement curves were converted into indentation (δ) curves and fitted to the Hertz model [17] (Eq. (1)) using Scanning Probe Imaging Processor (SPIP from Image metrology A/S Horsholm Denmark) and taking into account a spherical tip with a curvature radius of 20 nm (α). Finally, the Poisson ratio (ν) has been arbitrary fixed to 0.5.

$$F = \frac{4E\sqrt{R}}{3\left(1-\nu^2\right)}\delta^{3/2}. \quad (1)$$

Force curves were recorded in the force volume mode according to a matrix of 64 × 64 points on areas of 10 μm^2. For each force curve, Young's modulus is calculated and represented in false colors on elasticity maps. We also deduced height images from force volume measurements. The contact point of the force curves indeed indicates the sample height. It is therefore possible to deduce height images from a resoluted force volume experiment. AFM images and elasticity maps were analyzed using SPIP. We used OpenFovea (kindly provided by Charles Roduit and Sandor Kasas: http://www.freesbi.ch/openfovea) for the in depth analysis of the force curves.

2.2. Statistical analysis

Except when specified in the text or in legends, all data represent the mean ± S.E.M.. The number of independent experiments is indicated in the text or in the legend of the figures. Statistical significance of the data was assessed using unpaired 2-tailed Student's *t* test or ANOVA for repeated measures with Bonferroni or Tukey's post-hoc test. Non-quantitative data from TEM experiments were compared using Fisher's exact test. *P < 0.05, **P < 0.01, ***P < 0.001.

3. Results

3.1. Validation of cell surface topography imaging of living CMs using AFM modes

AFM studies were performed on non-beating living CMs isolated from adult young mice hearts as described in Supporting Material and Methods section and were conducted in three different imaging modes. To bypass CM surface deformation observed in contact mode (Fig. 1A), we used tapping mode making intermittent contacts with the CM surface (Fig. 1B) and allowing high resolution and direct

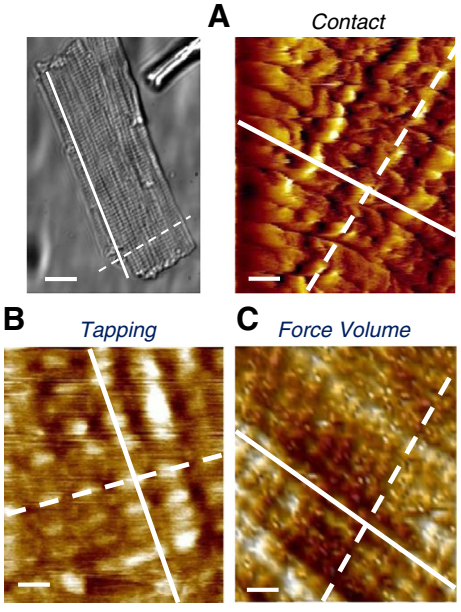

Fig. 1. Cell surface topography of living CMs obtained by AFM analysis. CMs were isolated from two-month old mice hearts. Representative images of living CMs surface were obtained using different AFM modes: contact (A), tapping (B) or force volume (C) as described in Material and Methods. Long and short CM axes are respectively depicted by the solid and dashed lines as indicated in the optical image. Bars: 2 μm.