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Remodeling of the transverse tubular system after myocardial infarction in rabbit correlates with local fibrosis: A potential role of biomechanics

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ABSTRACT

The transverse tubular system (t-system) of ventricular cardiomyocytes is essential for efficient excitation-contraction coupling. In cardiac diseases, such as heart failure, remodeling of the t-system contributes to reduced cardiac contractility. However, mechanisms of t-system remodeling are incompletely understood. Prior studies suggested an association with altered cardiac biomechanics and gene expression in disease. Since fibrosis may alter tissue biomechanics, we investigated the local microscopic association of t-system remodeling with fibrosis in a rabbit model of myocardial infarction (MI). Biopsies were taken from the MI border zone of 6 infarcted hearts and from 6 control hearts. Using confocal microscopy and automated image analysis, we quantified t-system integrity (I_{TT}) and the local fraction of extracellular matrix (f_{ECM}). In control, f_{ECM} was 18 ± 0.3%. I_{TT} was high and homogeneous (0.07 ± 0.006), and did not correlate with f_{ECM} ($R^2 = 0.05 \pm 0.02$). The MI border zone exhibited increased f_{ECM} within 3 mm from the infarct scar (30 ± 3.5%, $p < 0.01$ vs control), indicating fibrosis. Myocytes in the MI border zone exhibited significant t-system remodeling, with dilated, sheet-like components, resulting in low I_{TT} (0.03 ± 0.008, $p < 0.001$ vs control). While both f_{ECM} and t-system remodeling decreased with infarct distance, I_{TT} correlated better with decreasing f_{ECM} ($R^2 = 0.44$) than with infarct distance ($R^2 = 0.24$, $p < 0.05$). Our results show that t-system remodeling in the rabbit MI border zone resembles a phenotype previously described in human heart failure. T-system remodeling correlated with the amount of local fibrosis, which is known to stiffen cardiac tissue, but was not found in regions without fibrosis. Thus, locally altered tissue mechanics may contribute to t-system remodeling.

Contents

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Abbreviations

LCC	L-type Ca^{2+} channel
RyR	Ryanodine receptor
SR	Sarcoplasmic reticulum
T-tubules	Transverse tubules
T-system	Transverse tubular system
WGA	Wheat germ agglutinin
f_{ECM}	Fraction of extracellular matrix
I_{TT}	T-tubule integrity
d_{scar}	distance from infarct scar

1. Introduction

Excitation-contraction coupling in cardiac myocytes is the process by which electrical activation triggers mechanical contraction of the sarcomeres (Bers, 2002; Stern, 1992). The process involves opening of sarcolemmal voltage-gated L-type Ca^{2+} channels (LCCs) in response to membrane depolarization, leading to an initial influx of Ca^{2+}, which in turn triggers the opening of ryanodine receptors (RyRs) clustered in the membrane of the sarcoplasmic reticulum (SR). The SR serves as an intracellular Ca^{2+} store. As a result of RyR opening, Ca^{2+} is released from the SR into the cytosol, raising free cytosolic Ca^{2+} levels transiently from 100 to 200 nM at rest to approximately 1 μM. Ca^{2+} then binds to troponin C, inducing a conformational change in the troponin complex and rotation of tropomyosin. This enables actin-myosin interactions, which lead to sarcomere contraction. The contractile force developed by a myocyte depends on the number of contracting sarcomeres and force development in each sarcomere, which is regulated by the concentration of free cytosolic Ca^{2+} during systole. Half-maximal force is developed at about 600 nM (Gao et al., 1994; Stern, 1992; Yue et al., 1986). Obviously, efficient and forceful contraction of a myocyte requires a spatio-temporally homogeneous increase in free cytosolic Ca^{2+} by synchronized opening of RyRs.

For this purpose cardiomyocytes of humans and most other mammals possess a dense system of tubular membrane invaginations, the transverse tubular system (t-system). The membrane of transverse tubules (t-tubules) comes close to the SR membrane, forming junctions with a distance of only 10–12 nm between the sarcolemma and the SR (Forbes and Sperelakis, 1982). These junctions enable immediate and efficient coupling of RyR opening to Ca^{2+} influx through LCCs in response to membrane depolarization. In ventricular myocytes of human, canine, rabbit and other species, most RyRs are found within SR-sarcolemma junctions, but a smaller fraction of RyRs is more distant from the sarcolemma (including the t-system). They are commonly referred to as non-junctional RyRs. The distance between non-junctional RyRs and the sarcolemma ranges up to several micrometers (Dries et al., 2013; Jayasinghe et al., 2009; Torres et al., 2014). The ratio of junctional and non-junctional RyRs depends on species, preparation, method of analysis and the definition of “non-junctional”.

In cardiac diseases, especially in heart failure and myocardial infarction (MI), the t-system undergoes structural remodeling, resulting in alterations of shape and orientation as well as loss of t-tubules (Brette and Orchard, 2003; Ferrantini et al., 2013; Heinzel et al., 2008; Kostin et al., 1998; Louch et al., 2006; Lyon et al., 2009; Wei et al., 2010). These structural changes of the t-system in ventricular myocytes are gradual and differ between species. While t-system remodeling in rodents leads mainly to disorganized orientation and increased axial components (Chen et al., 2012; Wagner et al., 2012; Wei et al., 2010), the predominant remodeling in humans and larger mammals, such as canine or pig, is loss and dilation of t-tubules (Biesmans et al., 2011; Crossman et al., 2017; Kostin et al., 1998; Sachse et al., 2012). We recently described the 3D structure of sheet-like remodeled t-tubules (t-sheets) in humans, which are symptomatic for end-stage heart failure (Seidel et al., 2017). It still remains unclear if t-sheets are also present in animal models of heart failure, but most likely not in mice or rats because these species have already been studied extensively. In a porcine model of MI “enlarged, highly branched disordered structures” of the t-system were described in a recent study using three-dimensional electron microscopy (Pinali et al., 2017). These structures may correspond to the “sheet-like” components observed by confocal microscopy in human heart failure.

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