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EDITORIAL

T-tubular collagen: a new player in mechanosensing and disease?

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This editorial refers to 'Increased collagen within the transverse tubules in human heart failure' by D.J. Crossman *et al.*, pp. 879–891

In ventricular cardiomyocytes, excitation–contraction coupling is critically dependent on transverse-tubules (t-tubules). These specialized invaginations of the cell membrane penetrate deep into the cell interior, and interact with a variety of strategically located protein macromolecules to optimize the coordination of membrane signalling. During the action potential, Ca^{2+} entry through L-type Ca^{2+} channels within the t-tubules triggers the opening of closely apposed ryanodine receptors in the sarcoplasmic reticulum, thus eliciting a rise in cytosolic [Ca^{2+}] and contraction. Reduced efficiency of this process of Ca^{2+}-induced Ca^{2+} release is a hallmark of heart failure, and has been linked to disrupted t-tubular structure.[1,2] A number of studies have observed loss and/or reorganization of t-tubules under pathological conditions, which results in the formation of 'orphaned' ryanodine receptors, for which Ca^{2+} release is not directly triggered by L-type channel opening.[3] High resolution imaging has revealed that there are also more subtle changes in t-tubular structure during heart failure, including dilation of the tubule lumen, and the formation of sheet-like t-tubule structures.[4] Such nanoscale distortion of t-tubular organization may contribute to impaired Ca^{2+} release, by shifting L-type channels away from ryanodine receptors and impairing coupling. Furthermore, a larger t-tubule lumen augments diffusion of extracellular Ca^{2+} and K^{+}, which has been proposed to promote action potential prolongation and arrhythmia formation.[5]

Identifying the triggers for t-tubule remodelling during heart failure is an important goal, as it is hoped that preventing or reversing these changes might therapeutically strengthen the heartbeat. Loss of the t-tubule anchor junctophilin-2 has recently been linked to decreased overall t-tubule density in failing cardiac cells.[6,7] But what processes underlie t-tubule dilation? In the current issue of *Cardiovascular Research*, Crossman and colleagues[8] present intriguing data identifying a striking localization of collagen within the t-tubule lumen in failing cardiomyocytes. They specifically show that the presence of several collagen isoforms (I, III, IV, and VI) is closely paired to increased t-tubule diameter. Moreover, the authors observed that fibroblasts, which are well-established producers of interstitial collagen, extend filopodia into the t-tubule lumen. While the authors acknowledge that detailed studies are required to examine the time course of these changes during disease progression, their work raises the exciting hypothesis that collagen deposition within t-tubules drives their structural remodelling. If this is true, strategies aimed at decreasing fibrosis in the diseased heart might be found to have a rather unexpected benefit, by safeguarding nanoscale cardiomyocyte structure and function.

The findings of Crossman *et al.*[8] are also of interest for understanding cardiomyocyte mechanosensation. Even in healthy cardiomyocytes, they observed modest amounts of collagen within the interior of t-tubules, while dystrophin was localized on the t-tubule periphery. The dystrophin complex is known to link the extracellular matrix to the cytoskeleton, which enables force and strain transfer between the two compartments. The observation that collagen extends into the t-tubules, co-localizing with the dystrophin complex, provides important new insight into how mechanical stimuli might be sensed within these structures. Indeed, previous work has indicated that t-tubules deform during normal cardiomyocyte contraction,[9] and based on their localization along z-lines, t-tubules are likely well-positioned to transfer stress/strain to established mechanosensors in the z-disk. Deposition of the fibrillar collagen isoforms I and III during heart failure would be expected to stiffen t-tubule membranes. This effect could be particularly marked if local lysyl oxidase-mediated collagen crosslinking occurs under the direction of the proteoglycan syndecan-4.[10] It is unclear how mechanosensation might be altered within stiffened t-tubules that are resistant to deformation during cellular contraction. However, at the tissue level abnormal stress and strain across the heart have been linked to t-tubule loss.[7] Thus, an interesting possibility is that a collagen-laden, rigid t-tubule might be marked for degradation. While speculative, this premise would link collagen deposition not only to nanoscale t-tubular dilation, but also to the macroscale loss of t-tubules widely reported in heart failure. Such mechanisms would lend further support to the rationale for therapeutic unloading of the failing heart, either pharmacologically or via ventricular assist devices, which has been linked to reversal of both collagen deposition and t-tubule remodelling.[11]

Interestingly, Crossman also identified the presence of collagen IV and VI within t-tubules; isoforms which are less investigated in heart. Unlike collagen types I and III, these non-fibrillar isoforms are not believed to be important contributors to tensile strength and stiffness. Rather, collagen IV

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and VI have been linked to roles such as fibroblast migration and differentiation, and are suggested to anchor the fibrillar collagens to the basement membrane of cardiomyocytes. Indeed, collagen VI, which the authors have examined in particular detail, is believed to bind to a variety of integrins.[12] Thus, while deposition of collagen I and III during heart failure might be expected to stiffen t-tubules, anchoring of these isoforms may be maintained by a parallel increase in collagens IV and VI. In addition, it is possible that collagens IV and VI may cause direct signalling changes through their binding to integrins in macromolecular complexes.

Beyond heart failure, mechanosignalling via t-tubules may also be critically involved in other diseases, particularly those in which the components of collagen/dystrophin signalling complex are disrupted. For example, in Duchenne muscular dystrophy, lack of dystrophin protein is associated with increased membrane malleability in skeletal muscle.[13] In addition to skeletal muscle weakness and structural irregularities, many of these patients also develop cardiac abnormalities, and a recent study reported t-tubule disorganization in cardiomyocytes from the *mdx* mouse model.[14] Limb girdle muscular dystrophies, which include mutations in the sarcoglycans and dystroglycans, might be similarly expected to exhibit deficient t-tubule mechanosensing in both skeletal and cardiac muscle. But how will we ever understand these processes in detail? One way forward might be to perform detailed measurements of membrane flexibility in healthy and diseased myocytes,[13] paired with imaging of t-tubule geometry during the cardiac cycle.[9] In this way, we might link changes in t-tubule stiffness and deformation during disease states to specific disease outcomes. Mathematical models of t-tubule mechanics will also be needed, as these are presently lacking. Ultimately, however, direct proof will be needed, such as the demonstration that disrupting t-tubular properties impairs mechanosensing and normalizing diseased t-tubule properties restores it.

The Crossman findings are also to the best of our knowledge the first demonstration of a novel form of cardiomyocyte–fibroblast interaction. Cardiomyocyte–fibroblast interactions are known to be important in the normal and diseased heart. A variety of paracrine interactions between cardiomyocytes and fibroblasts promote heart failure, and electrical interactions can cause arrhythmias.[15] However, this is the first time that fibroblasts have been shown to directly cause collagen accumulation and structural derangements in cardiomyocyte organelles, with important potential functional consequences. These insights also point to a possible shortcoming of conducting experiments on isolated cardiomyocytes, for which functional regulation by fibroblasts is absent and t-tubular collagen may be stripped away by the collagenases employed during cell isolation procedures.

In conclusion, the present findings by Crossman and colleagues identifying collagen within the t-tubule lumen provide exciting new insights into how mechanosensation may occur within these structures, as well as how these structures are altered in disease. With mechanical linkage between the extracellular matrix and the cytoskeleton via the dystrophin complex, the t-tubules can be envisioned to serve as critical mechanosignalling hubs. Modulation of collagen deposition and t-tubule stiffness may regulate this signalling, while simultaneously remodelling t-tubular structure and function. This work opens the door to better understanding how cardiomyocytes dynamically respond to workload, and the multifaceted role of t-tubules in cardiac disease.

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