Subcellular Remodeling of the T-Tubule Membrane System

The Limits of Myocardial Recovery Revealed?

Article, see p 1632

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Impaired contractile function is the hallmark of systolic heart failure (HF). However, the exact mechanisms are not fully understood, and the resulting morbidity and mortality remain high despite significant advances in pharmacological and device therapies.[1,2] Resulting from plasticity of cardiac responses to injury, called pathological remodeling, the human heart changes in size, shape, and function. The culmination of complex, interdependent changes in molecular signatures, gene expression, metabolic adaptations, and cellular structures, the response of the failing heart to pathological insults of mechanical overload, neurohormonal excess, and ischemia has been studied extensively.[2] In addition, cardiomyocyte ultrastructural remodeling of subcellular organelles including sarcoplasmic reticulum (SR), sarcolemma (plasma membrane), mitochondria, myofibrils, and extracellular matrix has also been described during the development of HF.[3]

Communication between subcellular organelles is extensive in eukaryotic cells, involving plasma membrane, endoplasmic reticulum, mitochondria, nucleus, and other organellar structures through specialized membrane domains in close proximity to one another.[4] This communication relies on different protein entities, depending on the organelles involved, and controls key cellular processes in a spatial and temporal fashion including cell death, metabolism, and gene expression.[4] An example of interorganellar communication in cardiomyocytes is the important interaction of sarcolemma with SR during excitation-contraction (EC) coupling.[5,6] In this case, the transverse tubular system (T system) is the specialized membrane penetrating into the cardiomyocyte interior as an extensive network of T tubules which allows a rapid and synchronized transmission of the membrane action potential. Activation of L-type Ca^{2+} channels in the transverse tubule allows small amounts of Ca^{2+} into the cytosol, a quantity insufficient by itself to activate a contractile response. Rather, this Ca^{2+} acts as a messenger to trigger the release of Ca^{2+} from the SR to reach a critical [Ca^{2+}] threshold and initiate a contractile response.[5] This structural and functional Ca^{2+} microdomain (called couplon) comprises elements of the sarcolemma (≥1 voltage-gated L-type Ca^{2+} channels in the T system) and a group of type 2 ryanodine receptors (RyR2) in the SR.[5] This junction spans ≈12 nm.[7] Alterations of Ca^{2+} transients are a common feature of cardiomyocytes from failing myocardium and are thought to contribute to the progression of HF.[8] Moreover, in cardiomyocytes isolated from ventricles recovering contractile function by means of left ventricular assist device (LVAD) support, these abnormalities in Ca^{2+} cycling have been demonstrated to improve.[9]

Myocardial reverse remodeling is a complex phenotype marked by dynamic changes at multiple levels impacting cardiomyocyte contractile force and ventricular size and function.[10] When the therapy of maximal mechanical unloading is superimposed on a program of HF therapies proven to impact reverse remodeling, improvement in

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myocardial function can be significant, with a subset of these patients able ultimately to be weaned from LVAD support.[11] It is in this context that the groundbreaking study of Seidel and colleagues[12] in the current issue of *Circulation* has advanced our knowledge of subcellular membrane systems and their biological importance in myocardial remodeling (Figure).

In their previous work with a canine model of dyssynchronous heart failure, Li and colleagues[13] showed that T-system depletion was accompanied by increased abundance of nonjunctional RyR clusters uncoupled from the L-type Ca^{2+} channels.[13] Remarkably, applying cardiac resynchronization therapy to induce myocardial reverse remodeling, they found that the T-system depletion was reversible and associated with restoration of Ca^{2+} transients. In an extension of these studies into human HF, Seidel and colleagues used fluorescent staining, confocal microscopy, and 3-dimensional image analysis to investigate the T-system structure, density, and distance between RyR clusters and the sarcolemma in fixed myocardium, in isolated cardiomyocytes from unused donor hearts, and in chronic HF patients undergoing implantation of an LVAD.[12] They showed for the first time that: (1) a novel structural phenotype of the T tubules (a sheet-like T system rather than tubular membrane invaginations of the sarcolemma) in human cardiomyocytes from end-stage failing myocardium was associated with an increased distance separating RyR and the sarcolemma, heterogeneous intracellular Ca^{2+} release, and impaired EC coupling. (2) A tight relationship between the structure of the T system and the effectiveness of mechanical unloading in affecting recovery of function. High degrees of T-system remodeling (increased RyR sarcolemma separation) at the time of LVAD implantation were associated with absence of functional cardiac recovery during mechanical unloading, whereas preserved T-system structure was associated with recovery. Remarkably, the left ventricular ejection fraction in patients with pre-LVAD RyR-sarcolemma distances >1 μm did not improve following a period of mechanical unloading. This study has identified an important ultrastructural signature in human failing myocardium, and possibly a *sine qua non* of the end-stage failing heart.

Nevertheless, some key questions arise from this landmark study.

1. Do we need further confirmation that this pattern of the T-tubule system is a signature of the end-stage failing heart? If one could induce in an animal model a disrupted T-tubule system and compromise the functionality of this interconnected membrane system, would this result in progressive myocardial remodeling toward advanced stage HF? Alternatively, future studies using super-resolution imaging could corroborate the exact ultrastructural remodeling of the T-system with techniques such as stimulated emission depletion microscopy.
2. If sheet-like T-tubule remodeling is a necessary condition for defining the end-stage failing myocardium, is it also sufficient? That is, in patients with chronic cardiomyopathy at an earlier stage of the HF syndrome, does the presence of this T-system disruption imply obligate disease progression and portend inability of reverse remodeling with therapies that include mechanical unloading. Earlier intervention with modalities that reverse HF may be the key to providing a robust and durable recovery, because the study also found that the patients with the shortest duration of HF were more likely to have preserved T-system structure and function. In a recently convened National Heart, Lung and Blood Institute working group on myocardial recovery with mechanical unloading, a top priority included the study of mechanisms behind the durable remission from HF and how this could be achieved.[14]
3. If this pattern is confirmed to be that of an end-stage failing heart, do we have a structural basis for a more informed, personalized targeting of therapies for advanced HF? This novel phenotype of T-sheet ultrastructural remodeling of the membrane systems that regulate Ca^{2+} within the cardiomyocyte can be used for a tailored approach to maximize survival before and after advanced therapies in patients with presumed end-stage HF. The patients with advanced HF who have intact and preserved T-tubule structure may have the potential for myocardial recovery on a platform of mechanical circulatory support. In these patients, clinicians can apply maximal mechanical unloading along with standard HF therapies with the possible addition of experimental therapies to maximize the likelihood of myocardial reverse remodeling and achieve a remission or recovery from end-stage HF. This bridge to recovery may not be possible in the patients with sheet-like remodeling of the T-tubule membrane system, because patients with this ultrastructural signature demonstrated a very limited degree of myocardial reverse remodeling with mechanical unloading. It must be noted, however, that remodeling toward a more functional T-tubule system could still take place with other therapies that reverse HF but not, as Seidel and colleagues[12] have convincingly demonstrated, with left ventricular mechanical unloading. Given the evidence that both loading and unloading impact the structure and function of T tubules,[15] it is not surprising that therapies targeting the mechanical transduction of load may not reverse the subcellular abnormalities within the T system. Future work could determine whether therapies that lead to

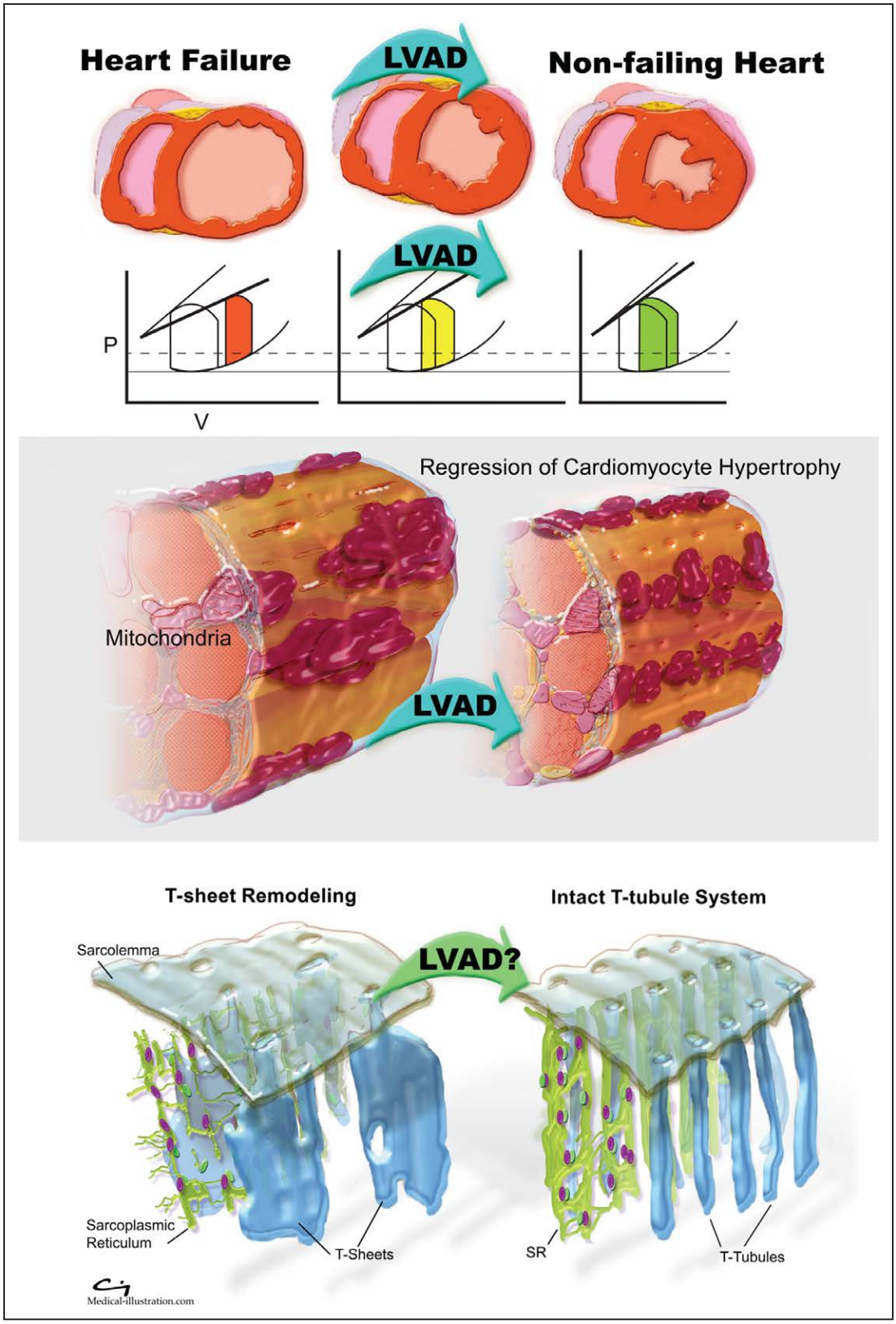

Figure. Left ventricular remodeling with mechanical unloading: considerations at the organ, cellular (tissue) and ultrastructural level.

Myocardial reverse remodeling in human heart failure after left ventricular assist device (LVAD) has been demonstrated with reductions in left ventricular end-diastolic and end-systolic dimension and improved mechanics resulting in increased slope of the end-systolic pressure volume relation (heavy line) and increased ventricular ejection and stroke volume. At the cellular (*Continued*)

Figure Continued. level, a regression in cardiomyocyte hypertrophy with a reduction cardiomyocyte cross-sectional area after a period of mechanical unloading with both pulsatile and continuous-flow devices has been confirmed by several studies. Electron microscopy of the cardiomyocyte ultrastructure has revealed sheet-like remodeling of the T-tubule membrane system (in blue) in patients with end-stage heart failure, resulting in a disruption of the coupling or dyad distance between the ryanodine receptor type 2 (RyR2, green) of the sarcoplasmic reticulum (also in green) and the L-type calcium channel (purple) of the T-tubular membrane, critical in maintaining calcium-induced calcium release and synchronous excitation-contraction coupling. Patients who have end-stage heart failure with this ultrastructural signature of detubulation into a T-sheet membrane system did not demonstrate myocardial reverse remodeling with a program of LVAD–induced mechanical unloading, raising the possibility that disruption of this membrane system to this degree precludes recovery of ventricular function with mechanical assist device support.

recovery of other deficits (metabolic, adrenergic receptor signaling, etc) and improve functionality also impact the remodeling of the T-system and couplon density.

4. Does this pattern of T-tubule system remodeling in the advanced stage of HF also occur in the less pressurized right ventricle? If so, can it provide a cellular biomarker helpful in predicting whether the right ventricle can withstand long-term LVAD support and allow survival without late right HF.

Irrespective of how these scenarios play out, this work by Seidel and colleagues has reinvigorated a fundamental question in human biology: how do we know a failing organ has reached end-stage, especially an organ such as the heart with functional capacity that is very load sensitive? In doing so, their work has illuminated the cardiomyocyte ultrastructure as an essential starting point for all to consider in elucidating the molecular basis of myocardial recovery.

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None.

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FOOTNOTES

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REFERENCES

1. Braunwald E. Heart failure. *JACC Heart Fail*. 2013;1:1–20. doi: 10.1016/j.jchf.2012.10.002.
2. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation*. 2013;128:388–400. doi: 10.1161/CIRCULATIONAHA.113.001878.
3. Dhalla NS, Rangi S, Babick AP, Zieroth S, Elimban V. Cardiac remodeling and subcellular defects in heart failure due to myocardial infarction and aging. *Heart Fail Rev*. 2012;17:671–681. doi: 10.1007/s10741-011-9278-7.
4. Bravo-Sagua R, Torrealba N, Paredes F, Morales PE, Pennanen C, López-Crisosto C, Troncoso R, Criollo A, Chiong M, Hill JA, Simmen T, Quest AF, Lavandero S. Organelle communication: signaling crossroads between homeostasis and disease. *Int J Biochem Cell Biol*. 2014;50:55–59. doi: 10.1016/j.biocel.2014.01.019.
5. Bers DM. Cardiac excitation-contraction coupling. *Nature*. 2002;415:198–205. doi: 10.1038/415198a.
6. Ibarra C, Vicencio JM, Estrada M, Lin Y, Rocco P, Rebellato P, Munoz JP, Garcia-Prieto J, Quest AF, Chiong M, Davidson SM, Bulatovic I, Grinnemo KH, Larsson O, Szabadkai G, Uhlén P, Jaimovich E, Lavandero S. Local control of nuclear calcium signaling in cardiac myocytes by perinuclear microdomains of sarcolemmal insulin-like growth factor 1 receptors. *Circ Res*. 2013;112:236–245. doi: 10.1161/CIRCRESAHA.112.273839.
7. Franzini-Armstrong C, Protasi F, Ramesh V. Shape, size, and distribution of Ca(2+) release units and couplons in skeletal and cardiac muscles. *Biophys J*. 1999;77:1528–1539. doi: 10.1016/S0006-3495(99)77000-1.
8. Bers DM. Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol*. 2014;76:107–127. doi: 10.1146/annurev-physiol-020911-153308.
9. Chaudhary KW, Rossman EI, Piacentino V 3rd, Kenessey A, Weber C, Gaughan JP, Ojamaa K, Klein I, Bers DM, Houser SR, Margulies KB. Altered myocardial Ca2+ cycling after left ventricular assist device support in the failing human heart. *J Am Coll Cardiol*. 2004;44:837–845. doi: 10.1016/j.jacc.2004.05.049.
10. Dipla K, Mattiello JA, Jeevanandam V, Houser SR, Margulies KB. Myocyte recovery after mechanical circulatory support in humans with end-stage heart failure. *Circulation*. 1998;97:2316–2322.
11. Birks EJ, George RS, Hedger M, Bahrami T, Wilton P, Bowles CT, Webb C, Bougard R, Amrani M, Yacoub MH, Dreyfus G, Khaghani A. Reversal of severe heart failure with a continuous-flow left ventricular assist device and pharmacological therapy: a prospective study. *Circulation*. 2011;123:381–390. doi: 10.1161/CIRCULATIONAHA.109.933960.
12. Seidel T, Navankasattusas S, Ahmad A, Diakos NA, Xu WD, Tristani-Firouzi M, Bonios MJ, Taleb I, Li DY, Selzman CH, Drakos SG, Sachse FB. Sheet-like remodeling of the transverse tubular system in human heart failure impairs excitation-contraction coupling and functional recovery by mechanical unloading. *Circulation*. 2017;135:1632–1645. doi: 10.1161/CIRCULATIONAHA.116.024470.
13. Li H, Lichter JG, Seidel T, Tomaselli GF, Bridge JH, Sachse FB. Cardiac resynchronization therapy reduces subcellular heterogeneity

of ryanodine receptors, T-tubules, and Ca2+ sparks produced by dyssynchronous heart failure. *Circ Heart Fail*. 2015;8:1105–1114. doi: 10.1161/CIRCHEARTFAILURE.115.002352.
14. NHLBI Working Group on Advancing the Science of Myocardial Recovery with Mechanical Circulatory Support. June 2 to June 3, 2016. http://www.nhlbi.nih.gov/research/reports/nhlbi-working-group-advancing-science-myocardial-recovery-mechanical-circulatory-support. Accessed April 12, 2017.
15. Ibrahim M, Terracciano CM. Reversibility of T-tubule remodelling in heart failure: mechanical load as a dynamic regulator of the T-tubules. *Cardiovasc Res*. 2013;98:225–232. doi: 10.1093/cvr/cvt016.

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