University of Utah
UNDERGRADUATE RESEARCH JOURNAL

CELLULAR STRUCTURES INVOLVED IN EXCITATION-CONTRACTION COUPLING OF THE HEART REMODEL WITH AGE

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Introduction

Heart disease continues to be the leading cause of death in the United States and accounts for one in every four deaths in patients 65 and over[4]. Heart failure is a specific heart disease where the heart weakens to the point that it cannot pump effectively, affecting approximately 7% of men and 5% of women over 60 the United States[1]. One underlying symptom of heart failure is remodeling of the transverse tubule system (t-system), ryanodine receptors (RyRs), and junctophilin-2 (JPH2). The t-system in cardiomyocytes is a system of inward folds in the membrane that create a tubular network (t-tubules)[5]. The t-tubules have voltage-gated calcium channels along their lengths, which allow calcium to enter the cell and attach to the RyRs on the surface of the sarcoplasmic reticulum (SR)[5]. This activates RyRs to allow calcium release from the SR into the cardiomyocyte, which facilitates contraction of the cell[2]. In a normal t-system, the RyRs on the SR and calcium channels in the t-tubules are kept proximal by JPH2, forming a unit called a couplon[6]. Couplons increase the speed at which calcium attaches to the RyRs[3,5]. A large number of couplons facilitates this calcium-induced calcium release, which leads to normal contraction[6]. The cardiomyocytes of a failing heart have a greater distance between RyR clusters and the t-tubules, fewer couplons, and inefficient excitation-contraction coupling[6].

Even though heart failure is more prevalent in older patients, we still do not understand the structural changes that the t-system, RyR, and JPH2 undergo with age, despite our understanding of the remodeling these constituents undergo in heart failure. To gain insight into this topic, we analyzed 3-dimensional (3D) reconstructions of t-tubules, RyR, and JPH2 clusters from confocal microscopic images of left ventricular myocytes from human donor hearts of varying ages.

Methods

Left ventricular myocardium samples were obtained from 13 human donor hearts rejected for transplantation due to noncardiac reasons. The age of the donors ranged from 20 to 69 years old. The donor tissue was snap-frozen in optimal cutting temperature compound and stored at -80°C. A cryotome was used to acquire 100 µm thick tissue sections, which were immediately fixed. We performed immunohistochemistry on the sections with mouse monoclonal ryanodine receptor (MA3-916, ThermoFischer Scientific) at 1:100 to label RyRs and rabbit polyclonal JPH2 (40-5300, ThermoFischer Scientific) at 1:100 to label JPH2 followed by their corresponding secondaries. CF555 wheat germ agglutinin (WGA, 29076, Biotium, Hayward, CA, USA) was used to label the t-system and membrane at 30 µg/ml. The tissue sections were then mounted on a glass slide using Flouromount-G (#17984-25, Electron Microscopy Science, Hatfield, PA, USA) and sealed with a coverslip. The sections were then imaged after a minimum of 24 hours using a Leica TCS SP8 confocal microscope (Leica, Gena, Germany).

Image stacks were acquired from regions of interest in each mounted tissue section, selected based on signal quality. Each stack was 100x100 µm and up to 30 µm thick to cover at least one

cardiomyocyte in its entirety. The resolution of each stack was 0.1x0.1x0.1 μm. Different signals from the same region were acquired by exciting with lasers of wavelengths corresponding to the antibodies of WGA, RyR, and JPH2.
Each image stack was then statistically analyzed using MATLAB (2019a, MathWorks, USA) to measure the mean distance between RyR clusters and t-tubules (ΔRyR), the mean distance between JPH2 clusters and t-tubules (ΔJPH2), RyR cluster density, and JPH2 cluster density.

Results
In young donors, the RyR clusters, JPH2 clusters, and t-tubules are numerous, and ordered. The three components also are consistently proximal. In older donors, RyR and JPH2 clusters are less numerous and less ordered than in younger donors. The distance between the RyR clusters and the t-tubules is greater in the older donors than in the younger donors. Similarly, the distance between the JPH2 clusters and the t-tubules is greater in older donors than in younger donors. The mean density of JPH2 clusters is also consistently higher in older donors. The mean density of RyR clusters is higher in older donors but has high variability.

Conclusion
The results suggest that distance between RyR and JPH2 clusters, and the t-tubules increases as a person ages and the mean density of RyR and JPH2 clusters decreases with age. These increases in distance and decreases in density indicate that there are fewer couplons, which results in less efficient excitation-contraction coupling. Fewer couplons as a result of remodeling is similar to heart failure remodeling. However, there is an absence of t-sheets, which is distinct from heart failure patients[6]. Clinical factors in the older patients do not indicate heart failure, but their heart tissue nonetheless undergoes remodeling, which suggests that the heart becomes less efficient as it ages. Remodeling in these structures in cardiomyocytes may be a contributing factor to the higher prevalence of heart failure in older patients.

Acknowledgments
This work was supported by funding from the Nora Eccles Treadwell Foundation, the American Heart Association, and the Summer Program for Undergraduate Research at the University of Utah (SPUR) awarded to Marcus Blackburn. We acknowledge Ms. Younjee Lee and Dr. Yankun Lyu, MD/PhD for their contributions to tissue imaging.

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