doi:10.1017/S1431927620016025
Microsc. Microanal. 26 (Suppl 2), 2020
© Microscopy Society of America 2020

Multiscale, Multimodal Imaging of Structure and Function Reveals Mechanisms of Normal and Abnormal Cardiac Physiology

Yusuf Olgar[1], Alec Miler[1], Heather Struckman[2], Rengasayee Veeraraghavan[1] and Przemyslaw Radwanski[1]

[1]The Ohio State University, Columbus, Ohio, United States, [2]The Ohio State University, Hilliard, Ohio, United States

While it has long been known that proteins that exist in close proximity influence each other's function, it is increasingly appreciated that there exist specialized structural nanoscale that act as functional units of cardiac biophysical phenomena. Notably, recent evidence from our group[1-3] and others[4, 5] suggests that a subset of Na_v channels reside in a close proximity to Ca^{2+} handling machinery (Ca^{2+} release channels, ryanodine receptors; RyR2, and Na^+/Ca^{2+} exchange; NCX). This suggest that these Na_v channels may contribute to the modulation of Ca^{2+} release. For instance, loss-of-function mutations in $Na_v1.1$ (Dravet Syndrome) have previously been shown to cause an increase in the incidence of epileptic seizures and cardiac arrhythmias[6, 7]. Interestingly, this loss-of-function mutation leads to an increase in Na^+ influx into the cardiomyocyte, thereby precipitating arrhythmias through Ca^{2+} handling dysregulation. Surprisingly, reduction in $Na_v1.6$ decreased the incidence of epileptic seizures, suggesting a possible link between $Na_v1.6$ and cardiac arrhythmias in Dravet Syndrome[8, 9]. Hence, we hypothesize that changes in $Na_v1.6$ functional expression in close proximity to Ca^{2+} handling machinery contributes to arrhythmias in $Na_v1.1$ haploinsufficiency.

Proximity ligation assay, which allows assessment of pairs of proteins that are within 40 nm, reveals that Dravet cardiomyocytes evidence an increase in the number of $Na_v1.6$-RyR2 and $Na_v1.6$-NCX proximity points along the transverse-tubules relative to wild type cardiomyocytes. Additionally, Stochastic Optical Reconstruction Microscopy (STORM) in Dravet hearts reveals an increases in $Na_v1.6$ clusters within 50 nm of RyR2, but not further away, suggesting that remodeling within these nanodomains may contribute to increased Na^+ influx and the resulting abnormal Ca^{2+} handling in these mice.

To gain insight into the functional consequences of nanodomain remodeling in Dravet, we first performed scanning ion conductance microscopy (SICM)-guided patch clamp. This approach allows positioning of patch clamp pipet with 20-40 nm precision on cardiomyocytes surface. SICM-guided patch clamp revealed an increase in local Na_v activity within the cardiomyocyte transverse-tubules and not in other regions. This increase in local Na_v activity was coupled to increased aberrant Ca^{2+} release in the form of Ca^{2+} sparks. Additionally, Dravet cardiomyocytes evidenced increased Ca^{2+} wave incidence. These abnormalities in Ca^{2+} handling translated into increased ventricular tachycardia incidence *in vivo*. Consistent with increased $Na_V1.6$ in Dravet mice, reduction in $Na_v1.6$, either pharmacologic or through genetic means, resulted in a reduction in aberrant Ca^{2+} release on cellular level and arrhythmia burden *in vivo*.

https://doi.org/10.1017/S1431927620016025 Published online by Cambridge University Press

In summary, multiscale, multimodal imaging of structure and function uncovers $Na_V1.6$ remodeling within transverse-tubules in a $Na_V1.1$ haploinsufficiency. Importantly, these structural changes correlated with increased Na_V activity, Na_V-mediated Ca^{2+} handling dysfunction and increased ventricular tachycardia incidence. These results suggest that remodeling within Na^{+}/Ca^{2+} handling nanonodomains may contribute to the development of ventricular tachycardia.

References

[1] PB Radwański et al. *Cardiovasc Res* **106** (2015), p. 143.
[2] PB Radwański et al. *JACC Basic Transl Sci* **1** (2016), p. 251.
[3] HL Struckman et al. *Microsc Microanal* (2020), p. 1.
[4] SKG Maier et al. *Circulation* **109** (2004), p1421.
[5] RE Westenbroek et al. *J Mol Cell Cardiol* **64** (2013), p. 69.
[6] DS Auerbach et al. *PloS One* **8** (2013), e77843.
[7] F Kalume et al. *J Clin Invest* **123** (2013), p. 1798.
[8] MS Martin MS et al. *Hum Mol Genet* **16** (2007), p . 2892.
[9] LL Anderson et al. *Sci Rep* **7** (2017), p. 1682.

https://doi.org/10.1017/S1431927620016025 Published online by Cambridge University Press