University at Buffalo *The State University of New York* | REACHING OTHERS

The Role of Sestrin2 in Regulating Cardiomyocyte Contractility

Marissa Boniszewski, Alexander Morrison-Nozik, Si Chen, Dr. Ji Li

[1]*Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo (SUNY)*

Introduction

Understanding the molecular mechanisms of impaired cardiac contractility after ischemia reperfusion insults is important for the development of novel therapeutic strategies. Recently, a novel protein Sestrin2 has been implicated in response to hypoxia because of its ability to accumulate in the rat brain during hypoxia and protect cell viability when given hypoxia and nutrient depletion (Budanov et al., Oncogene, 2002). Moreover, Sestrin2 has the ability to act as an antioxidant under oxidative stress by reducing reactive oxidation species (ROS) levels, providing a key mechanism in preventing cell death in reperfusion (Budanov et al., Science, 2004).

Sestrin2 has also been shown to activate the enzyme AMP-activated protein kinase (AMPK) under stressful conditions (Budanov and Karin, Cell, 2008). Our lab and others have shown that AMPK can protect against myocardial infarction by becoming activated under stress, making it a promising therapeutic target for ischemic heart disease (Russell et al., J Clin Invest, 2004).

The fact that Sestrin2 can act as an antioxidant and also activate AMPK makes it an attractive protein to study during myocardial ischemia. There are multiple cell types in the heart expressing Sestrin2 one of the most important being cardiomyocytes that are responsible for the contractile function of the entire heart. The effect of Sestrin2 on AMPK and specifically its effect on mammalian cardiomyocytes has not yet been investigated. It is hypothesized that Sestrin2 plays an important role in maintaining cardiomyocyte contractility and integrity during hypoxic conditions.

Methods

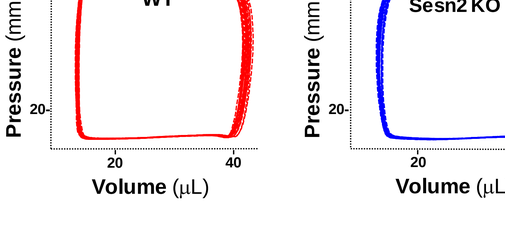
WT and Sestrin2 KO mice were anesthetized and the hearts were excised, cannulated and perfused in a Langendorff fashion with oxygenated Ca^{2+} free KHB buffer with butanedione monoxime and then digested with oxygenated Ca^{2+} free KHB buffer with butanedione monoxime containing 0.067mg/mL Liberase Blendzyme 4 (Hoffmann-La Roche Inc.). The heart was perfused for about 20 minutes then removed from the system, minced and checked for 80% viability. Calcium was restored to the cells in increments to a final concentration of 1mM. The cells were then centrifuged and suspended in contraction buffer (Hepes buffer with glucose and calcium) with Fura-2 and incubated for 20 minutes.

After 20 minutes the Fura-2 was washed out and the cells were suspended in contraction buffer with sodium cyanide to chemically induce hypoxia at a low concentration of 500nM. After incubating the cells for 20 minutes the sodium cyanide was washed out and the cells were resuspended in contraction buffer.

Using a Leica microscope and Ionoptix software, the cardiac contractility and calcium influx of the cells were measured. The parameters measured include: the resting sarcomere length, the maximal velocity of shortening and relengthening, the peak height or difference between the baseline and peak values, peak shortening or the percent change during the transient, time to 90% peak shortening, baseline calcium signal, peak height of the calcium signal, and the peak shortening of the calcium signal.

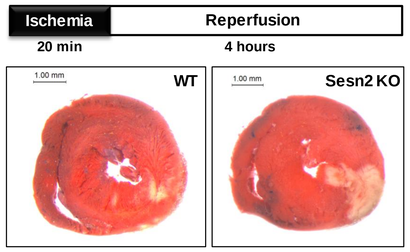
Results

1 Sestrin2 is expressed in adult cardiomyocytes.

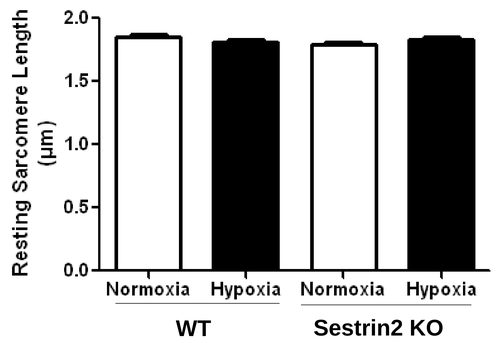


2 Under basal conditions, there is no significant difference in adult Sestrin2 KO and WT heart phenotype.

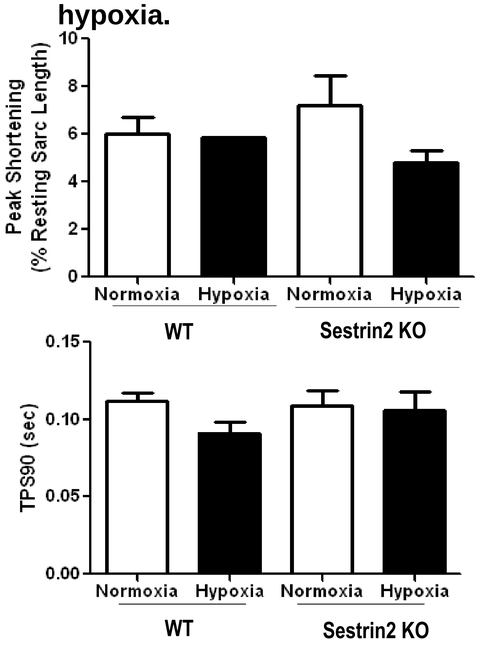
3 Myocardial Infarction is larger in Sestrin2 KO hearts.

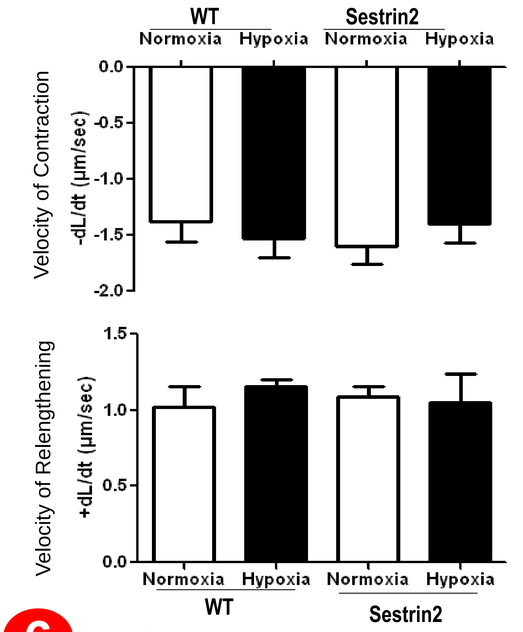


4 There is no difference in the resting sarcomere length between WT and Sestrin2 KO mice under hypoxic and basal conditions.

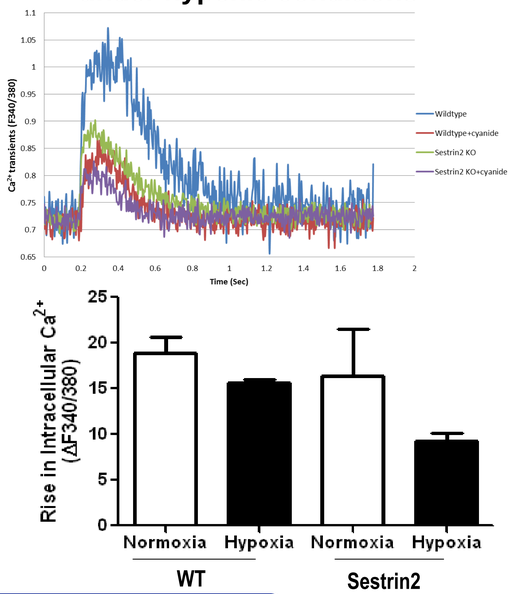


5 Sestrin2 KO cardiomyocytes display a trend of impaired cardiac contractility during hypoxia.





6 Ca^{2+} influx in Sestrin2 KO cardiomyocytes is impaired under hypoxic conditions



Conclusions

- Sestrin2 is expressed in adult cardiomyocytes showing phenotypic differences only under ischemic conditions.
- Cardiomyocyte contractility is impaired in Sestrin2 KO cardiomyocytes during hypoxia.
- The mechanism of impaired contractility may be due to impaired calcium influx.