Reciprocal Interactions of STIM1 with Orai1 and L-type Ca^{2+} Channels in Cardiac Myocytes

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Abstract

Pathological cardiac hypertrophy can be triggered by abnormal Ca^{2+} levels. It has been shown that the mechanisms governing context-dependent changes in Ca^{2+} influx are linked to stromal interaction molecule 1 (STIM1). STIM1 is a sarcoplasmic reticulum Ca^{2+} sensor that regulates Ca^{2+} influx by directly activating store operated calcium channels such as Orai1 in response to stress such as intracellular Ca^{2+} depletion. STIM1 is also known to regulate L-type Ca^{2+} channels in cardiomyocytes, though the mechanism has not been elucidated. To address this, we monitored STIM1 interactions with either Orai1 or L-type Ca^{2+} channels in neonatal rat ventricular cardiomyocytes (NRVMs) and adult rat ventricular cardiomyocytes (ARVMs) using the Duolink in situ fluorescence assay. These channel interactions were observed under endogenous culture conditions as well as thapsigargin (TG)-mediated calcium store depleted conditions. Differences in STIM1 interactions between control (untreated) and thapsigargin-treated NRVM and ARVM cells were observed. In NRVM cells, STIM1 interactions with Orai1 increased by 44.79 ± 2.68% when treated with thapsigargin as compared to the control population (n=73-83). Conversely, STIM1 interactions with L-type Ca^{2+} channels decreased by 52.31 ± 3.45% compared to the control (n=64-69). In ARVM cells, STIM1 interactions with Orai1 decreased by 59.19 ± 2.39% when treated with thapsigargin as compared to the control population (n=11-16). STIM1 interactions with L-type Ca^{2+} channels on the other hand increased by 74.13 ± 0.21% compared to the control (n=11-19). We observed an opposite trend in STIM1 interactions with Orai1 and L-type Ca^{2+} channels in NRVM and ARVM cells. When treated with thapsigargin to deplete calcium levels, their degree of interaction changed significantly. At a molecular level, it would seem that STIM1 is a versatile Ca^{2+} channel regulator as it can alternately interact with either Orai1 or L-type Ca^{2+} channels.

Hypothesis

The Ca^{2+}-sensing protein, STIM1, facilitates Ca^{2+} influx through molecular interactions with plasma membrane channels in neonatal and adult cardiac myocytes.

Methods

Duolink ® In Situ – Fluorescence

Duolink assay enables the detection, visualization, and quantification of protein interactions in cell and tissue samples using microscopy. This technique utilizes proximity ligation assay technology to directly monitor interactions between proteins within 40nm, which yields a fluorescent signal.[2]

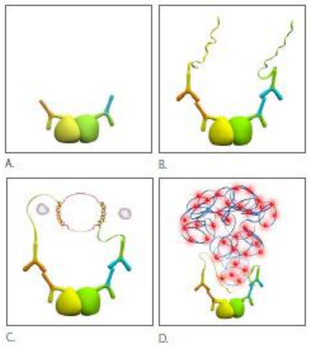

Figure 1. Duolink Assay Principle[2]

A. The samples were incubated overnight at 4ºC with primary antibodies (Orai1-Rb/STIM1-Mo & LCC-Mo/STIM1-Rb).
B. Secondary antibodies conjugated with oligonucleotides (PLA probe Mouse-MINUS and Rabbit-PLUS) were added to the reaction and incubated at 37ºC.
C. The Ligation solution, consisting of ligase and two oligonucleotides (illustrated as red bands), hybridized to the two PLA probes and are joined by the ligase to form a closed circle product. This was incubated at 37ºC for 30 minutes.
D. The Amplification solution consisted of polymerase, nucleotides and fluorescently labeled oligonucleotides. The oligonucleotide arm of one of the PLA probes acts as a primer for a rolling-circle amplification reaction using the ligated circle product as a template which generates a concatemeric (repeated sequence) product. Then, the fluorescently labeled oligonucleotides will hybridize to the RCA product and create a signal that is easily visible as a distinct fluorescent spot.

Results

STIM1 Interactions within Neonatal Cardiac Myocytes

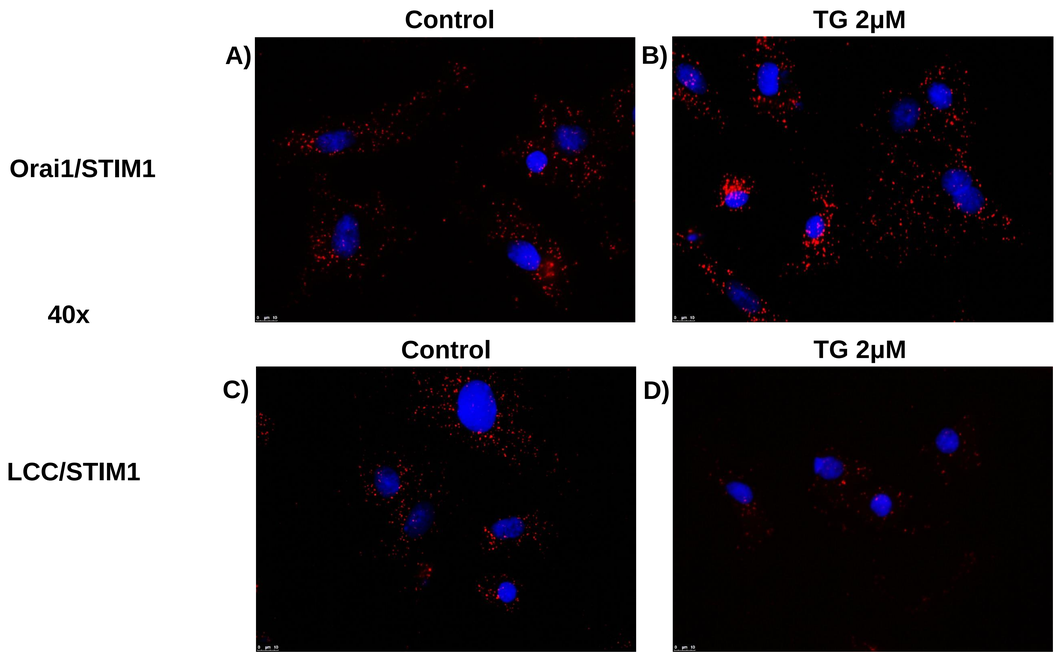

Figure 2. Qualitative Analysis of STIM1 Interactions with Orai1 & L-Type Ca^{2+} Channels in Neonatal Cardiac Myocytes
Representative images showing STIM1 interactions with Orai1 channels (A,B) and L-Type Ca^{2+} channels (C,D). Interactions are shown as red puncta. Nuclei are shown as blue. Images were obtained at exposure of 400.00 ms/intensity of 5 for red and exposure of 70.00 ms/intensity 4 for blue. Images were taken on DM5500 Leica confocal microscope.

STIM1 Interactions within Adult Ventricular Cardiac Myocytes

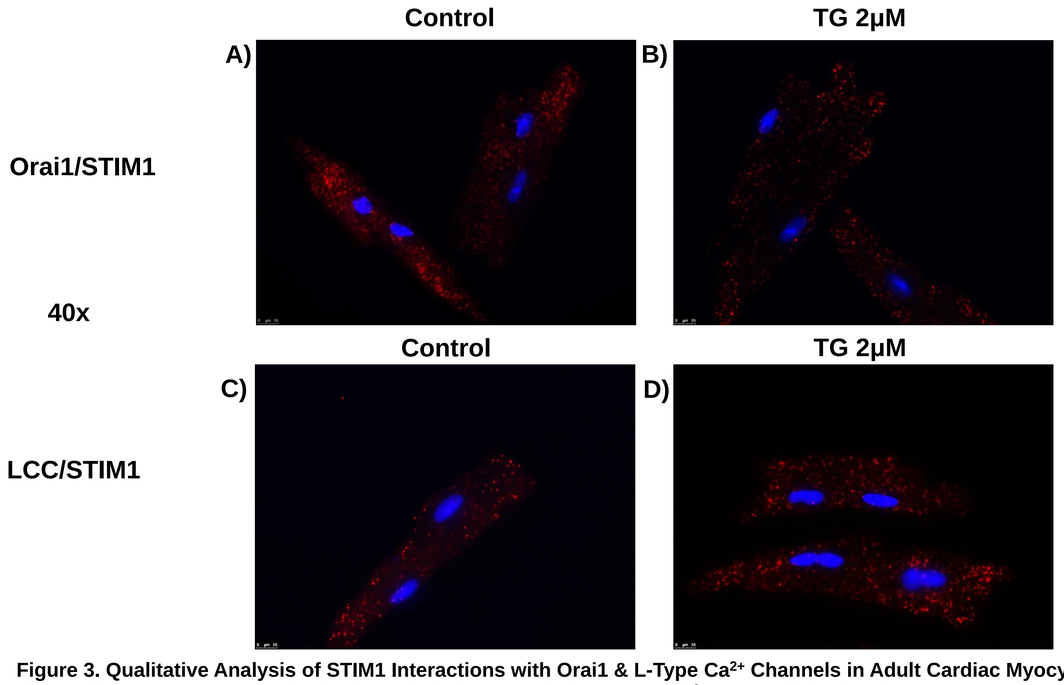

Figure 3. Qualitative Analysis of STIM1 Interactions with Orai1 & L-Type Ca^{2+} Channels in Adult Cardiac Myocytes
Representative images showing STIM1 interactions with Orai1 channels (A,B) and L-Type Ca^{2+} channels (C,D). Interactions are shown as red puncta. Nuclei are shown as blue. Images were obtained at exposure of 400.00 ms/intensity of 5 for red and exposure of 70.00 ms/intensity 4 for blue. Images were taken on DM5500 Leica confocal microscope.

Results Cont.

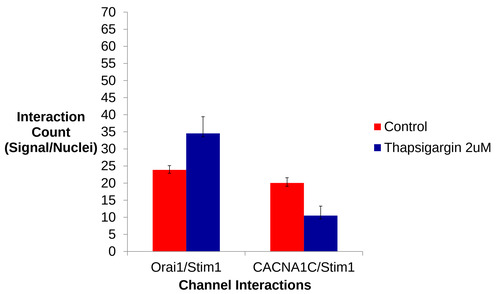

Figure 4. Quantitative Analysis of STIM1 Interactions in Neonatal Cardiac Myocytes
Interaction count depicts the number of fluorescent spots detected per nuclei in neonatal cardiomyocytes for Orai1 and L-type Ca^{2+} channels with STIM1. Images were obtain from four random areas on the 12 mm coverslips and quantified. Values from each individual area were totaled and averaged together to get representative value for the number of protein interactions per cell. Error bars represents the standard deviations for each average interaction count.

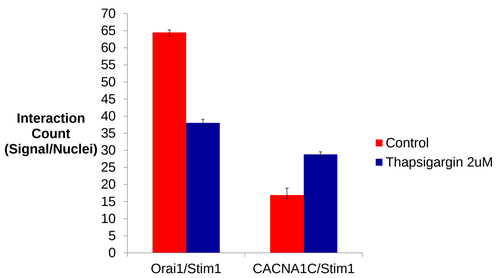

Figure 5. Quantitative Analysis of STIM1 Interactions in Adult Cardiac Myocytes
Interaction count depicts the number of fluorescent spots detected per nuclei in adult cardiomyocytes for Orai1 and L-type Ca^{2+} channels with STIM1. Images were obtain from four random areas on the 12 mm coverslips and quantified. Values from each individual area were totaled and averaged together to get representative value for the number of protein interactions per cell. Error bars represents the standard deviations for each average interaction count.

Conclusions

- Orai1 and L-type channels are observed to have an opposite trend when comparing STIM1 interactions within neonatal and adult cardiac myocytes.
- These data seem to suggest that STIM1 acts a versatile Ca^{2+} channel regulator in cardiac myocytes.
- Further studies into STIM1 protein interactions will aid the development of novel therapeutic strategies for the treatment of cardiac hypertrophy and disease.

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- Members of Hill Lab

References

1. Luo, Xiang, et al. "STIM1-dependent store-operated Ca^{2+} entry is required for pathological cardiac hypertrophy." *Journal of Molecular and Cellular Cardiology* 52.1 (2011): 136-47
2. Sigma Aldrich. *Duolink using PLA Technology*. Web. July 2014