The Increased Susceptibility of the Sarcospan-deficient Myocardium to beta-adrenergic Agonists Occurs Through Distinct Immune-mediated Mechanisms

Ian Marreel[1], Bolade S. Olateju[1], Nazanin Mohammadipoor[1], Ariella Barbati[1], Grace Mercier[1], **Michael Dadlani**[1], Michelle S. Parvatiyar[1]

1. Department of Health, Nutrition and Food Sciences, Florida State University, Tallahassee, FL, USA

ABSTRACT

Introduction: Sarcospan (SSPN) is a tetraspanin-like protein and integral member of the dystrophin-glycoprotein complex (DGC). Pathogenic variants in DGC proteins cause membrane leakiness, muscle weakness, and cardiac dysfunction. SSPN-deficiency causes a milder phenotype; however, under baseline conditions global SSPN-/- mice have a heightened fibrotic response compared to wild-type (WT). Our proteome data indicates that global SSPN-/- hearts have significant elevations of pro-inflammatory molecules under baseline conditions. Previously, it was shown that SSPN-/- mice have widespread myocardial damage and fibrosis after isoproterenol treatment (0.8 mg/day/two weeks) (Parvatiyar et al. JAHA, 2015). Since macrophages are dominant infiltrating immune cells in the injured myocardium, we examined the response of SSPN-/- bone marrow-derived macrophages (BMDM) to innate immune triggers. Compared to WT, SSPN-/- BMDM produced sustained levels of IL-1b in response to lipopolysaccharide (LPS), a priming step for the cytosolic pathogen recognition receptor NOD-like receptor protein 3 (NLRP3) inflammasome. **This led us to hypothesize that reduced membrane stability renders the SSPN-/- myocardium more vulnerable to beta-adrenergic stress through increased NLRP3 inflammasome activation.**

Methods: Male and female SSPN-deficient mice were treated with isoproterenol (0.8 mg/kg for 5 days) or sterile saline and tissues collected after 5 days. The hearts were evaluated for histological alterations and immunofluorescence was used to visualize immune cells including CD68+ macrophages, neutrophils (Ly6G+), and apoptosis-associated Speck-like protein containing a CARD (ASC) to assess (NLRP3) inflammasome activation. Membrane leakiness was evaluated by the presence of IgM+ fibers. Bone marrow cells were harvested from WT and SSPN-/- mice and differentiated in GM-CSF-containing media and stimulated with innate immune ligands (3- and 6-hours) and NLRP3 inflammasome activators (LPS and Nigericin).

Results: Histological examination revealed significant patchy fibrosis in isoproterenol-treated SSPN-/- hearts that correlated with areas of NLRP3 activation. Isoproterenol treated SSPN-/- hearts exhibited increased IgM+ cardiac myocytes suggesting subthreshold membrane instability that is increased in cardiac injury. BMDM experiments showed that SSPN-/- BMDM have a significantly elevated Type I interferon (IFN-I) response to B-DNA and LPS. As observed in the SSPN-/- myocardium, SSPN-/- BMDM also exhibited increased NLRP3 inflammasome activation

Conclusions: Our results suggest that the increased fibrosis in isoproterenol-treated SSPN-/- hearts may be the result of tissue injury due to heightened NLRP3 inflammasome activation. Further studies are needed to determine the cell types responsible for NLRP3 activation in the injured SSPN-/- myocardium.

INTRODUCTION

Sarcospan (SSPN) is a tetraspanin-like protein with an important role in stabilizing muscle cell membranes and facilitating cellular adhesion. SSPN expression is most abundant in striated skeletal and cardiac muscle, however it is expressed in many other tissues. Overall, global SSPN-deficient mice exhibit a mild phenotype and normal lifespan. However, under stress conditions such as beta-adrenergic stimulation SSPN-deficient mice exhibit heightened myocardial damage and fibrotic deposition (Parvatiyar, JAHA 2015).

In this study, we examine the abundance of several immune cell types in the SSPN-deficient hearts after beta-adrenergic stimulation and compare the response of bone-marrow derived macrophages obtained from these mice.

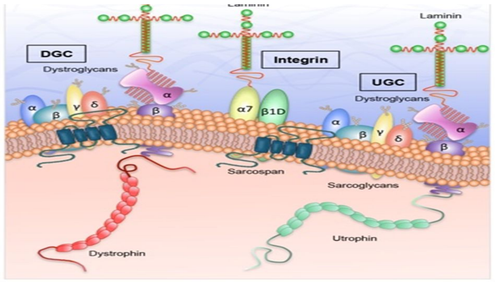

Figure 1. Sarcospan (SSPN) is an integral member the dystrophin-glycoprotein complex (DGC) and utrophin-glycoprotein complex (UGC). SSPN also associates with a7/B1D integrin (Parvatiyar et al. 2019)

RESULTS

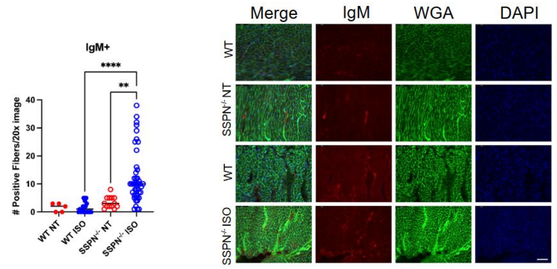

Figure 2. Increased membrane permeability in SSPN-deficient hearts following β-adrenergic stress. Representative immunofluorescence images of cardiac tissue from wild-type (WT) and SSPN$^{-/-}$ mice under non-treated (NT) or Isoproterenol (ISO) conditions. Sections were stained for IgM (red) to detect membrane permeability, WGA (green) to outline cardiomyocyte membranes, and DAPI (blue) to label nuclei. Merged images show localization of signals. Quantification of IgM-positive fibers per 20× field is shown in the left panel. SSPN$^{-/-}$ hearts treated with isoproterenol exhibit a significant increase in IgM-positive cardiomyocytes compared with WT controls and untreated groups, indicating enhanced membrane leakiness following β-adrenergic stress. Statistical significance is indicated (**p < 0.01, ****p < 0.0001).

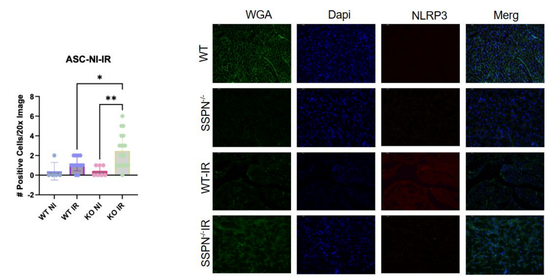

Figure 3. Enhanced inflammasome activation in SSPN-deficient hearts following cardiac injury. Representative immunofluorescence images of cardiac tissue from wild-type (WT) and SSPN$^{-/-}$ mice under non-injured (NI) and injury (IR) conditions. Sections were stained with WGA (green) to outline cardiomyocyte membranes, DAPI (blue) to label nuclei, and NLRP3 (red) to detect inflammasome-associated signaling. Merged images illustrate the spatial localization of these markers. Quantification of ASC-NI-IR–positive cells per 20× field is shown in the left panel. SSPN$^{-/-}$ hearts subjected to injury demonstrate a significant increase in inflammasome-positive cells compared with WT controls and non-injured groups, indicating enhanced activation of the NLRP3 inflammasome in the absence of Sarcospan. Statistical significance is indicated (*p < 0.05, **p < 0.01).

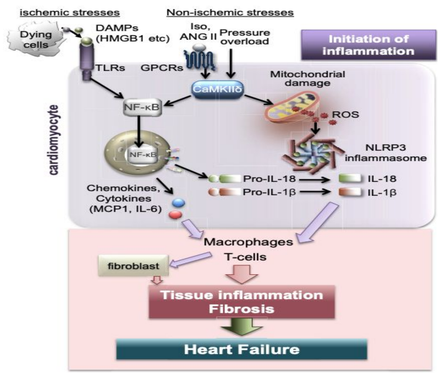

Figure 4. Cardiac Inflammation is initiated in cardiomyocytes in response to ischemic and nonischemic stress. In response to ischemic stress, damage-associated molecular patterns (DAMPs) released from dying cells activate Toll-like receptors (TLRs) leading to NF-kB activation and inflammation. Nonischemic stresses include ANG II, pressure overload and beta-adrenergic agonists (isoproterenol). Activation of NLRP3 inflammasome in cardiomyocytes and ROS production leading to production of IL-1β and IL-18. Figure from Suetomi et al. AJP Heart Circ. Physiol (2019).

Does increased IL-1β production prime the NLRP3 inflammasome in the heart after isoproterenol treatment?

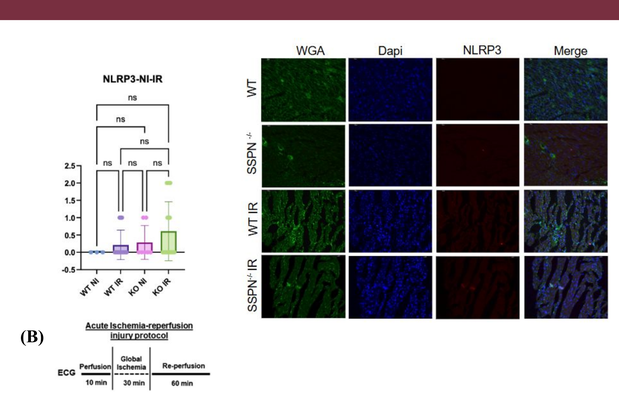

Figure 5. NLRP3 expression in wild-type and SSPN-deficient hearts following acute ischemia–reperfusion injury. Representative immunofluorescence images of cardiac tissue from wild-type (WT) and SSPN$^{-/-}$ mice under non-injured (NI) and ischemia–reperfusion (IR) conditions. Tissue sections were stained with WGA (green) to outline cardiomyocyte membranes, DAPI (blue) to label nuclei, and NLRP3 (red) to assess expression of the inflammasome-associated protein NLRP3. Merged images show the spatial relationship between these signals. Quantification of NLRP3-positive cells per 20× field is shown in the left panel. Although NLRP3 staining appears elevated in injured hearts, statistical analysis revealed no significant differences among the groups (ns). The schematic below (B) illustrates the acute ischemia–reperfusion protocol consisting of baseline perfusion, 30 minutes of global ischemia, and 60 minutes of reperfusion.

CONCLUSIONS

- Loss of Sarcospan compromises cardiomyocyte membrane stability, as demonstrated by increased IgM-positive fibers in SSPN$^{-/-}$ hearts following β-adrenergic stress.
- SSPN deficiency is associated with enhanced inflammatory signaling, including increased activation of the NLRP3 inflammasome in injured myocardium.
- SSPN$^{-/-}$ bone marrow–derived macrophages exhibit heightened innate immune responses to inflammatory stimuli, suggesting immune cells may contribute to the amplified inflammatory environment.
- Although ischemia–reperfusion injury did not significantly increase total NLRP3 expression, the overall data support increased inflammasome activation rather than simple protein abundance as a driver of pathology.
- Collectively, these findings suggest that SSPN protects the myocardium from stress-induced injury, and its loss sensitizes the heart to inflammation and fibrosis through enhanced inflammasome signaling.