Abstract: **P1629**

**Crispr/Cas9 to elucidate cardiac specific effects of Gdf11**

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**Topic(s):**
Basic Science - Cardiac Biology and Physiology: Signal Transduction, Mechano-Transduction

**Citation:**
Background: Heart failure with preserved ejection fraction (HFpEF) is a prevalent clinical condition in the aging population. HFpEF lacks specific therapies with a significant impact on survival, different therapeutic approaches have indeed failed, indicating a specific need in understanding the pathophysiology of the disease. Cardiac hypertrophy is one of the main features of HFpEF that contributes to impair diastolic function. Reducing ventricular stiffness associated with cardiomyocyte hypertrophy may indeed result in improved diastolic filling. Growth differentiation factor 11 (Gdf11), a TGF-β family factor, has been identified as a circulating factor able to reduce cardiac hypertrophy.

Purpose: Similar to myostatin (Gdf8), Gdf11 promotes the activation of atrophy pathways that induce ubiquitination of sarcomeric proteins. Gdf11 and Gdf8 activate both type I and II Tgf-β receptors, specifically by interacting with Acvr2a and Acvr2b (type II) and Alk 4/5/7 (type I), they activate Smad 3/4 pathway. Our data indicate a specific and more potent effect of Gdf11 in reducing cardiomyocytes size that is not recapitulated by Gdf8. Understanding the specific effect of Gdf11 on cardiomyocytes is crucial to develop therapeutic strategies to target the hypertrophic phenotype.

Methods: To investigate the effects of specific type I receptor KO on Smad signaling cascade we performed our preliminary experiment on HL-1 cells, a cardiac muscle cell line carrying a doxycycline inducible Cas9 transgene. Cell sensitivity of HL-1 to Gdf11 and Gdf8 was tested by performing a dose-response curve using a luciferase reporter for Smad 3/4 pathway activation (CAGA12). Selective Tgf-β type I receptor KO was induced using, for each receptor, two sgRNAs that have been designed to cleave the receptor coding sequence creating INDEL mutations and disrupt proper translation of the protein and confirmed by western blotting. Smad 3/4 activity was measured using a CAGA12-luciferase assay on HL-1-Cas9 transduced cells.

Results: Our data in HL-1 cells confirm a more potent effect of Gdf11 in activating Smad 3/4 pathway when compared to Gdf8 (Fig. 1A). Selective Alk4 and Alk5 KO induced a similar reduction in Smad 3/4 activation for both Gdf11 and Gdf8. Interestingly, Alk7 KO significantly reduced Gdf11 signaling that was not recapitulated when using Gdf8, suggesting that Alk7 receptor is crucial for Gdf11-dependent Smad 3/4 activation in HL-1 cells (Fig. 1B).

Conclusions: Our preliminary results indicate that part of Gdf11 cardiac specificity when compared to Gdf8 may reside in the usage of Alk7 for signaling. Alk7 has proposed as a protective factor for pathological cardiac hypertrophy by negatively regulating Mek-Erk1/2 signalling. Our results are now under investigation in vitro using mouse neonatal cardiomyocytes expressing Cas-9 and in vivo using cardiac-specific-Cas9-expressing C57 transgenic mice, in resting condition and after induction of pathological hypertrophy.
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