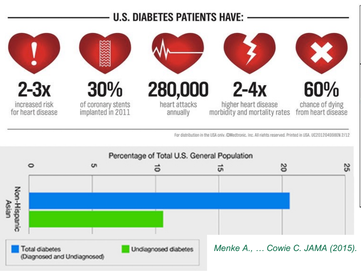
Sustained Cardiac Protein O-GlcNAcylation and Epigenetic Mechanisms of Cardiac Hypertrophy in a Diabetic-like State

Samuel F. Chang, CM Ha, S Bakshi, LA Potter, JC Chatham, AR. Wende
Medical Scientist Training Program, UABSOM. Dept. of Pathology, UABSOM

MEDICAL SCIENTIST TRAINING PROGRAM

Introduction

Global diabetes rates are nearing 10% (WHO), and the U.S. CDC has shown that diabetes correlates with a 2-4 times higher rate of heart disease morbidity and mortality. Additionally, more than half of Asian Americans with T2 Diabetes remain undiagnosed.



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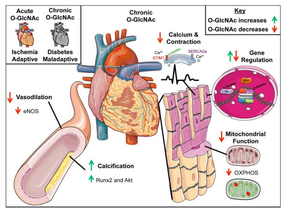

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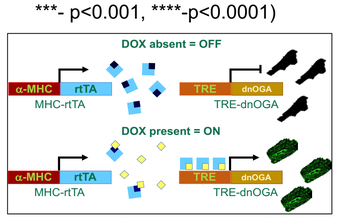
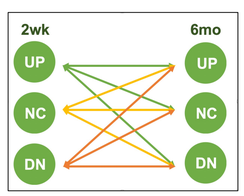
Our laboratory studies diabetes and heart failure and has focused on the posttranslational β-linked N-acetylglucosamine (O-GlcNAc) modification to serine/threonine residues (i.e. O-GlcNAcylation), as a key regulator of cardiovascular function and disease. O-GlcNAc is the product of the hexosamine biosynthetic pathway which is upregulated via increased flux of metabolic intermediates in hyperglycemia and diabetes. Specifically, others have shown that Calcium/Calmodulin dependent protein Kinase type IIβ (CaMKIIβ) upregulation due to O-GlcNAcylation causes increased reactive oxygen species (ROS) production, which when elevated contributes to cardiac arrhythmia, fibrosis, and apoptosis; NADPH oxidase (NOX), contributes to ROS production.

Objective: This study aimed to test the hypothesis that elevated GlcNAc levels regulate transcriptional changes that may initially be adaptive (2-weeks) but progress to dysfunction (6-months).

Methods

- Doxycycline (DOX)-inducible, cardiac specific (αMHC-rtTA), dominant negative O-GlcNAcase (dnOGA) mouse model (dnOGAh) to recapitulate O-GlcNAc induction by inhibiting its removal.
- At the given timepoints heart tissue was isolated, frozen, and then RNA was purified and used to perform RNA sequencing.
- After pre-processing and alignment, R software (v4.0.2) was used to identify differentially expressed genes by DESeq2 package.
- qPCR analysis to confirm the changes in gene expression.
- One-way and two-way ANOVA conducted: (* - p < 0,05, ** - p <0,01, ***- p<0.001, ****-p<0.0001)





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Results

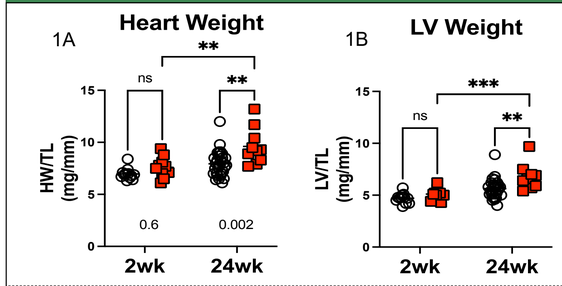


Figure 1: Cardiac Hypertrophy in chronic O-GlcNAC Hearts. A) Total heart weights are significantly increased in chronic O-GlcNAC hearts compared to controls and 2wk O-GlcNAC hearts. **B**) Left ventricle weights are significantly increased in chronic O-GlcNAC hearts compared to controls and 2wk O-GlcNAC hearts.

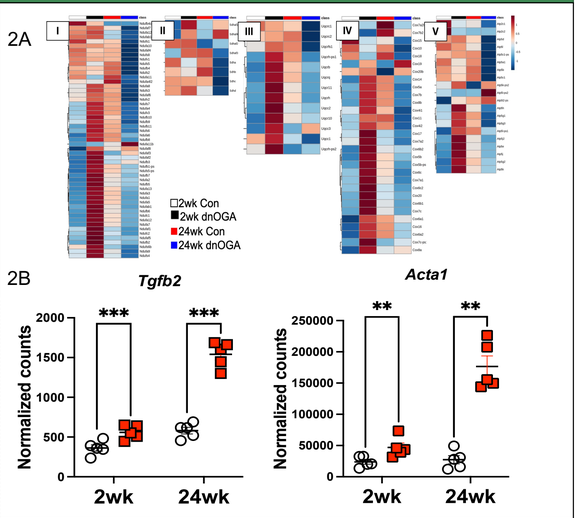
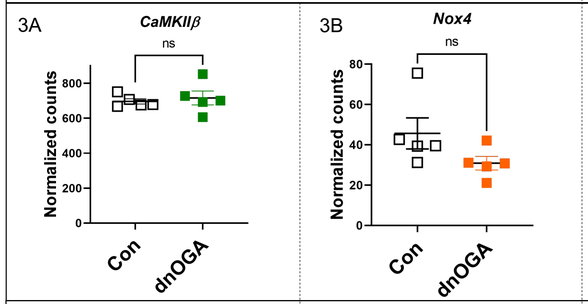


Figure 2: Acute protein O-GlcNAcylation enhances Ox. Phos. Pathways, but chronic protein O-GlcNAcylation induces cardiac fibrosis. A) ETC complex associate gene analysis shows increased Ox. Phos. in 2wk dnOGAh induction. **B**) Analysis of genes associated with fibrosis (Tgfb2, Acta1) are significantly upregulated in O-GlcNAC hearts compared to control; with increase change in the chronic state.



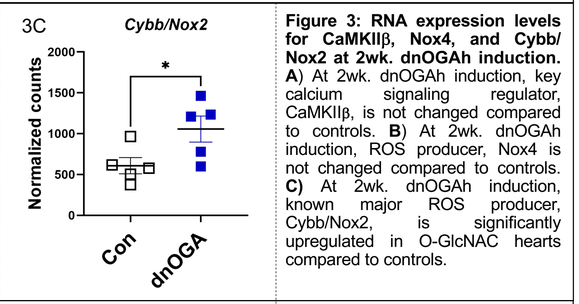
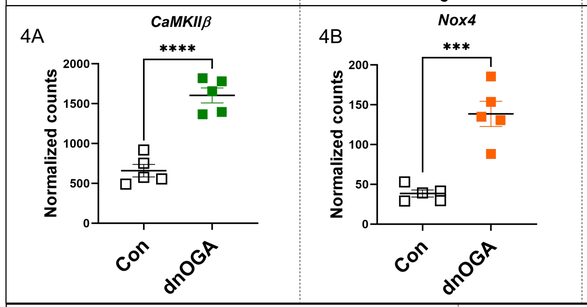


Figure 3: RNA expression levels for CaMKIIβ, Nox4, and Cybb/Nox2 at 2wk. dnOGAh induction. A) At 2wk. dnOGAh induction, key calcium signaling regulator, CaMKIIβ, is not changed compared to controls. **B**) At 2wk. dnOGAh induction, ROS producer, Nox4 is not changed compared to controls. **C)** At 2wk. dnOGAh induction, known major ROS producer, Cybb/Nox2, is significantly upregulated in O-GlcNAC hearts compared to controls.



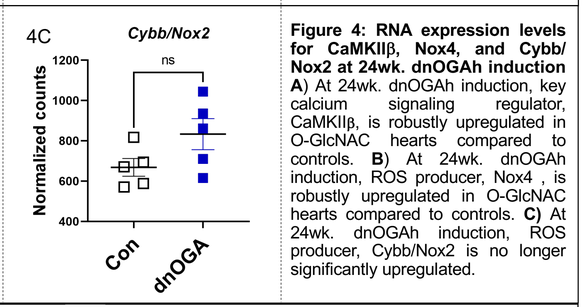


Figure 4: RNA expression levels for CaMKIIβ, Nox4, and Cybb/Nox2 at 24wk. dnOGAh induction A) At 24wk. dnOGAh induction, key calcium signaling regulator, CaMKIIβ, is robustly upregulated in O-GlcNAC hearts compared to controls. **B**) At 24wk. dnOGAh induction, ROS producer, Nox4 , is robustly upregulated in O-GlcNAC hearts compared to controls. **C)** At 24wk. dnOGAh induction, ROS producer, Cybb/Nox2 is no longer significantly upregulated.

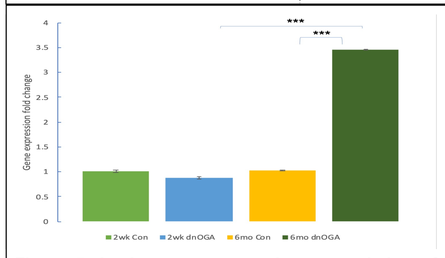


Figure 5: In-vivo gene expression upregulation of Nox4. in-vivo gene expression of Nox4 via PCR recapitulates results from in-silico analysis of Nox4.

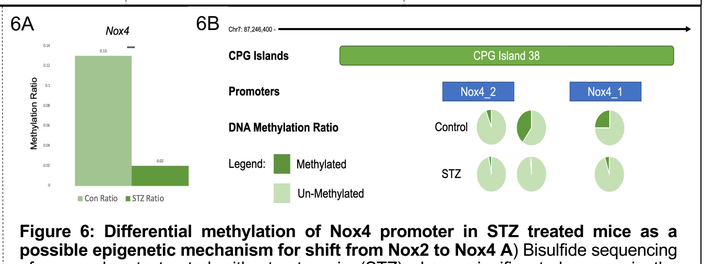
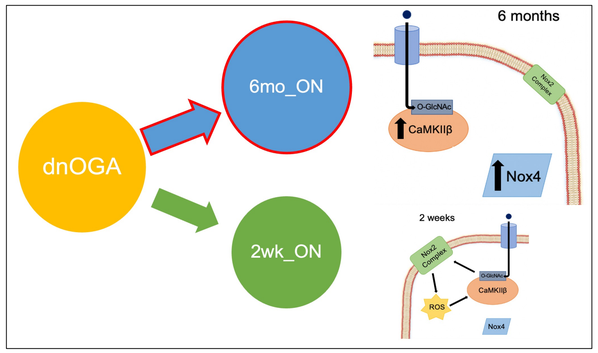


Figure 6: Differential methylation of Nox4 promoter in STZ treated mice as a possible epigenetic mechanism for shift from Nox2 to Nox4 A) Bisulfide sequencing of mouse hearts treated with streptozocin (STZ) shows significant decrease in the methylation at Nox4 **B**) Schematic representation of where the differentially methylated sites are located: within CPG islands, and at the known Nox4 promoter sites.

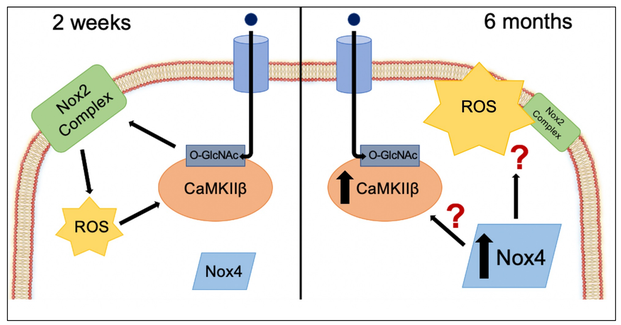
Conclusions

- Robust upregulation of Nox4, in parallel to CaMKIIβ, during chronic dnOGA induction strongly indicates a significant role in cardiac pathophysiology
  - Increased CaMKIIβ protein O-GlcNAcylation during periods of acute hyperglycemia, leading to ROS production via Nox2.
  - Modest Nox2 upregulation at 2wk, but robust Nox4 upregulation at 6m of O-GlcNAc induction.



Future Directions

- Elucidate the role/mechanism of Nox4 upregulation in the chronic O-GlcNAc hearts through a combination of in-silico, in-vitro and in-vivo studies
- Better understand the pathophysiology of diabetic cardiac dysfunction and therapeutically target these pathways to improve the prognoses of millions of individuals with diabetes.



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