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Title:

Bulk RNA Seq identifies activation of oxidative stress regulating glucose/lipid metabolism as potential mechanism of increased glucose sensitivity under conditional knockdown of HMGB1 in a Type-2 diabetes model

Introduction

Type 2 diabetes (T2D) has become one of the most prevalent diseases in the US. T2D is characterized by hyperglycemia, hyperinsulinemia, and systemic inflammation. High mobility group box 1 (HMGB1) is a pro-inflammatory protein that expresses ubiquitously in most tissues. Our previous work has shown that in a Streptozotocin (STZ) induced T2D mouse model, conditional knockdown of HMGB1 significantly decreased hyperglycemia by increasing glucose sensitivity and regulating mitochondria biogenesis through AKT/FoxO1 signaling. However, the metabolic mechanisms that guide how decreased HMGB1 drives this phenomenon remain unknown. We hypothesize that conditional knockdown of HMGB1 will activate genes that regulate oxidative stress in glucose/lipid metabolism under a STZ induced T2D model.

Method

Total RNA was extracted using Trizol (Invitrogen) from liver and muscle of inducible (iHMGB1) KO and HMGB1 flox mice that developed T2D after STZ injection (25mg/kg) following manufacture instructions. Isolated RNA was then reversely transcribed into cDNA using RNA to cDNA EcoDry Premix (TaKara). Key markers of glucose and glycogen metabolism including Glycogen synthase kinase (GSK), Phosphoribulokinase (PRK), Insulin Receptor β (IR- β), Protein kinase B (AKT), Forkhead box protein O1 (Foxo1) were quantified by reverse transcriptase PCR (RT-PCR). Genes were then quantified by RNA sequencing measurement of 3 HMGB1 Flox and 3 iHMGB1 KO liver samples. Differential expression analysis was performed comparing KO vs Flox. We identified 84 up and 25 downregulated genes by FDR=5% and fold-change ≥ 1.5 cutoff. These genes were further served as input for pathway analysis. Significant pathways were defined by FDR=5%.

Results

Liver RT-PCR showed FoxO1, PRK, and GSK to be downregulated in iHMGB1 KO mice with no significant changes in IR- β and AKT. However, in muscle, RT-PCR suggested a more substantial decrease in expression of Foxo1, PRK, GSK as well as IR- β and AKT. These findings indicated that muscle appears to be more sensitive to insulin and has an important role in maintaining glucose homeostasis under conditional HMGB1 knockdown. Bulk RNA-seq liver analysis showed that conditional HMGB1 knockdown alters metabolic pathways involved in glucose and lipid metabolism, most significantly by NRF2-mediated Oxidative Stress Response ($-\log(p\text{-value}) = 1.00E+01$), FXR/RXR Activation ($-\log(p\text{-value}) = 8.60E+00$) and LXR/RXR Activation ($-\log(p\text{-value}) = 5.00E+00$).

Conclusion

Results from RT-PCR and bulk RNA Seq analysis suggest that conditional knockdown of HMGB1 primarily alters oxidative stress pathways that have roles in enhancing glucose/lipid homeostasis. Future studies will be aimed at using tissue-specific conditional knockout of HMGB1 to further understand the distinct role that HMGB1 plays in each tissue under T2D phenotype.

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