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Using RNA Sequencing To Study B-Cell Biology

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Introduction

Type II Diabetes (T2D) is a complex disease that is influenced by environmental factors such as diet and life style as well as genetic variations that result in deviations in gene expression. Recent genome-wide association studies (GWAS) have established the correlation of more than 70 genetic variants (Single nucleotide polymorphisms or SNPs) with susceptibility to T2D [1,2]. Using highly efficient RNA sequencing (RNA-Seq) approach, it is now possible to comprehensively profile the transcriptome of the islets of Langerhans or individual β cells to better understand how these environmental factors and SNPs contribute to the pathogenesis of type II Diabetes. Next generation sequencing (NGS) technology has revolutionized the field of transcriptomics. RNA-seq is the current gold standard which has overcome the short comings of microarray analysis by extending its range of detection to low expressed genes, spliced variants and novel transcripts [3,4].

This technique is now being applied to the studies of islet biology and the understanding of T2D pathogenesis. Such studies confirmed some of the known characters such as signature gene expression of GCG (glucagon), DPP4 (Dipeptidyl peptidase 4) and GC (Vitamin D-binding protein) in α cells. Also it provided information of transcriptome profiling in rare endocrine cell type such as γ and δ cells. GHSR (Growth hormone secretagogue receptor) was specifically expressed in δ cells and γ cell that also express genes such as SERTM1 (serine rich and transmembrane domain containing 1) ABCC9 (ATP binding cassette subfamily C member 9) and SLIT (slit guidance ligand). Other interesting findings include sub clustering within α, β and acinar cells. For example, a small subset of α cell expressing more proliferative genes was identified and a group of acinar cells expressing more inflammatory related genes were separate from the others.

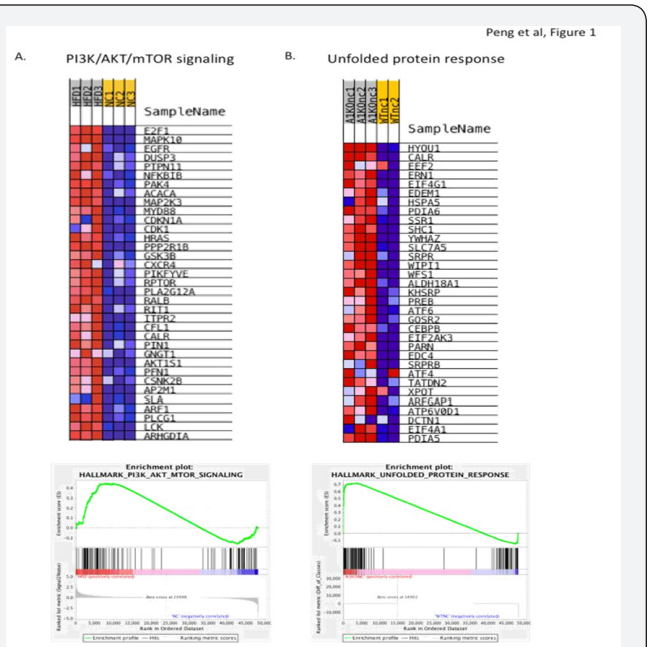
Furthermore, new genes correlated with T2D were also identified in a cell-specific manner. FXYD2 (FXYD domain containing ion transport regulator 2) encodes a gamma subunit of an Na, K-ATPase was confirmed to have a low expression only in T2D pancreatic β cells. Other genes upregulated in T2D β cells includes GPD2 (glycerol-3-phosphate dehydrogenase 2) and LEPROTL1 (Leptin receptor overlapping transcript-like 1). Negative regulators of glucose stimulated insulin secretion (GSIS) - RGS4 (regulator of G-Protein signaling 4) and CHRM3 (cholinergic receptor muscarinic 3)- were enriched in α cells. WFS1 (Wolframin ER transmembrane glycoprotein) is significantly decreased in T2D α cells [5].

Direct sequencing on dispersed or FACS sorted single cells have been developed and provided new understanding on the transcriptome differences among the cell types in islets of Langerhans. A recent study performed single-cell RNA-seq analysis on 609 non-diabetics and 883 T2D α, β, δ and PP cells. The authors identified 245 T2D related genes with 28% of which have no previously known functions. The authors also compared mouse α and β cells versus humans and found similar expression profiles. This study provided one of the first databases on single-cell transcriptomes of α, β, δ and PP cells that can be used to study functions of the newly identified genes [6].

In another study where RNA-seq was performed on FACS sorted endocrine cells using HIC1-2B4, a pan-endocrine marker, four different transcriptome profiles are identified among β cells. These four subsets are separated based on two markers: CD9 and ST8SIA1 (alpha-N-acetylneuraminide alpha-2,8-sialyltransferase), and named β1-4 as CD9-ST8SIA1-, CD9+ST8SIA1-, CD9-ST8SIA1+ and CD9+ST8SIA1+ respectively. RNA-seq on these 4 subsets of β cells indicated shared genes such as PDX1 (pancreatic and duodenal homeobox 1), INS (insulin) and MAFA (MAF BZIP transcription factor A) as well as unique genes such as HCN1 (hyperpolarization activated cyclic nucleotide gated potassium channel 1) in β 1/2 cells. In healthy

human islets, β1 subset has highest percentage among all β cells followed by β2, β3, and β4 subgroup. Importantly, among type 2 diabetic patients, this composition pattern was disrupted and particularly, the ST8SIA1+ β cells (β3 and β4) are abnormally high.

The author also provide evidence showing β3 and β4 subsets are less responsive to glucose stimulation, indicating its potential relevance to type 2 diabetes [7]. This finding is supported by another study where islets from six health subjects and four T2D patients were sequenced at single cell level and five different expression clusters were identified according to their transcriptome and different expression levels of RBP4 (retinol binding protein 4), FFAR4/GRP120 (free fatty acid receptor 4), ID1, ID2, and ID3 (inhibitor Of DNA binding, HLH protein) [5]. Using RNA-seq, we recently performed transcriptome analysis of mouse islets fed high fat diet (HFD) vs. normal chow diet (NC). Our study indicated that HFD caused enrichment of PI3K/AKT/mTOR pathway genes that contribute to adaptive increase in growth and proliferation in β cells in response to HFD insult (Figure 1A).



Figures 1A & 1B: GSEA (Gene set enrichment Analysis) analysis showing heat map and enrichment plot of A. genes in PI3k/AKT/mTOR pathway enriched by HFD in islets of Wild type mice. B. Unfolded protein responsegenes enriched by AKT1 deletion. NC-Normal chow, HFD- High fat diet, A1KO-AKT1 knock out.

Using mouse models lacking PTEN in the islets where PI3K/AKT signal is constitutively active, we have shown previously that this pathway is important for maintaining the mass of the islet β cells [8,9]. Furthermore, we showed that this ability of PTEN/PI3K signal to control β cell growth is dependent on their ability to regulate β cell senescence and how it interacts with the mesenchymal cells that supports the growth of islets [10,11]. Moreover, our recent study discovered AKT1 deficiency increases the UPR (unfolded protein response) signaling in β cell which potentially poised β cell to apoptosis caused by high fat diet (Figure 1B). Together, these studies suggest PI3K/AKT signaling is one of the key signaling in β cells that contributes to its growth and cell survival.

Our finding is supported by another similar RNA-Seq experiment in cultured human islets treated with palmitic acid for 48 hours [12]. The transcriptome profile indicated strong metabolic stress upon treatment and how β cell failure may have happened in response to this stress. Among the 1,325 genes modified by palmitate treatment, genes involved in fatty acid metabolism and endoplasmic reticulum (ER) stress signaling are highly enriched, including 11 out of 59T2D candidate genes. Whether these genes are also altered by loss of AKT or upregulation of PTEN remains to be elucidated. B cell transcription factors such as PDX1, MAFA, MAFB (MAF BZIP transcription factor B), NEUROD1 (Neuronal Differentiation 1), PAX4 (Paired Box 4) and GATA6 (GATA Binding Protein 6) were found to be repressed by this treatment.

Conclusion

In summary, the use of RNA-Seq approaches has provided new direction for diabetes research and allowed researcher to develop novel hypothesis to explore the pathogenesis of diabetes. The expression profile analysis also allowed more detailed classification of β cell to be identified and linked to T2D. Using the combination of RNA-seq with molecular pathogenesis analysis, we and have started to unveil the molecular mechanism for how HFD contributing to the pathogenesis of T2D.

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