Quantifying the Proteome and Acetylome In Diabetic Nephropathy: HPLC-MS/MS analysis of formalin-fixed paraffin-embedded tissue

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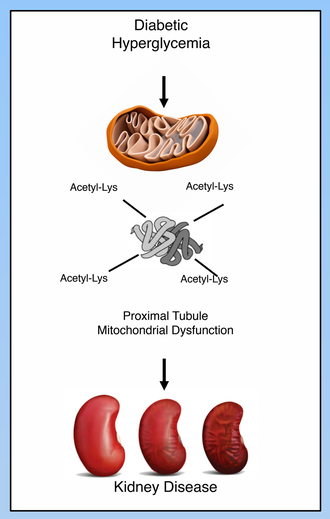
Introduction

Diabetes is currently the seventh leading cause of death in the United States. According to the Center for Disease Control (CDC), approximately 30.3 million people have diabetes. An astounding 84.1 million adults are believed to have prediabetes, where 90% of those individuals remain undiagnosed. Numerous health complications arise from diabetes, including nephropathy. Diabetic nephropathy (DN) is defined by both structural and functional changes of the kidneys; including mesangial expansion, glomerular basement membrane thickening, and podocyte injury. Progression of DN can ultimately lead to chronic kidney disease (CKD), end-stage renal disease, and kidney failure. Although there are well-known structural changes of DN at the glomerulus, alterations occurring at the proximal tubule are often overlooked. Recent findings suggest that persistent damage at the proximal tubule contributes to the progression of CKD. A potential mechanism underlying proximal tubule dysfunction is the result of aberrant post-translational modifications (PTM). One such modification of particular interest is lysine acetylation, which is regarded as a metabolic footprint PTM. Dysregulated acetylation can result in altered protein function. The overall goal of this study is to advance our understanding regarding how protein acetylation is distorted during diabetic hyperglycemia and how it can ultimately play a role in the pathogenesis of diabetic kidney disease.



Scheme 1. Preliminary data demonstrate that diabetic hyperglycemia can promote altered lysine acetylation at the proximal tubule of the kidneys that can lead to mitochondrial dysfunction, which may play a pivotal role in the progression of diabetic nephropathy.

Hypothesis

Diabetic kidney disease alters the renal proteome and impacts protein acetylation due to metabolic disruptions, resulting in impaired mitochondrial function.

Methods

Received human kidney tissue curls from diabetic cases (n=6) and non-diabetic control cases (n=9) preserved in formalin-fixed paraffin-embedded (FFPE) from collaborators at John Hopkins University. Each case had three tubes containing three 10 μm curls. Tissues were deparaffinized at room temperature using heptane. Total protein extraction for each tube containing sample was carried out according to Qproteome FFPE Tissue Kit (Qiagen, Hilden, Germany). Sonication cycles were incorporated into the Qproteome method to facilitate total tissue solubilization. RC/DC protein assay (Bio-Rad, Hercules, CA) was used to determine total protein quantification. Triplicate samples were combined per case for a total of 6 diabetic samples and 8 non-diabetic controls and were subjected to in-solution tryptic digestion using 8M urea lysis buffer. Digestion occurred overnight at room temperature. Digestion was quenched using 20% TFA and samples were purified using Sep Pak column (Waters, Milford, MA). Peptides were stored at -80C in 5% ACN + 0.1% TFA until prepared via lyophilization for total peptide and acetylomic analysis using an Agilent 6550 QTOF. The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 [http://david.abcc.ncifcrf.gov] was utilized to identify biologically enriched pathways within the protein list.

Results

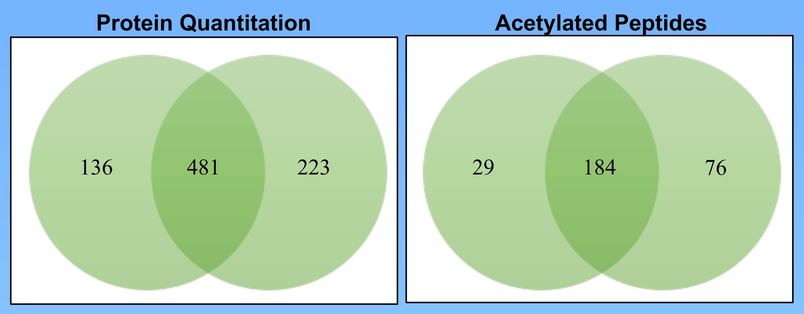


Figure 1. (A) A total of 840 proteins were identified from proteomic analysis. Of these, 136 proteins were significantly down-regulated and 223 were up-regulated in the diabetic tissue as compared with the non-diabetic control samples. (B) A total of 289 proteins were identified in the acetylomic analysis. 76 were significantly down-regulated and 29 were significant up-regulated (p-value < 0.05)

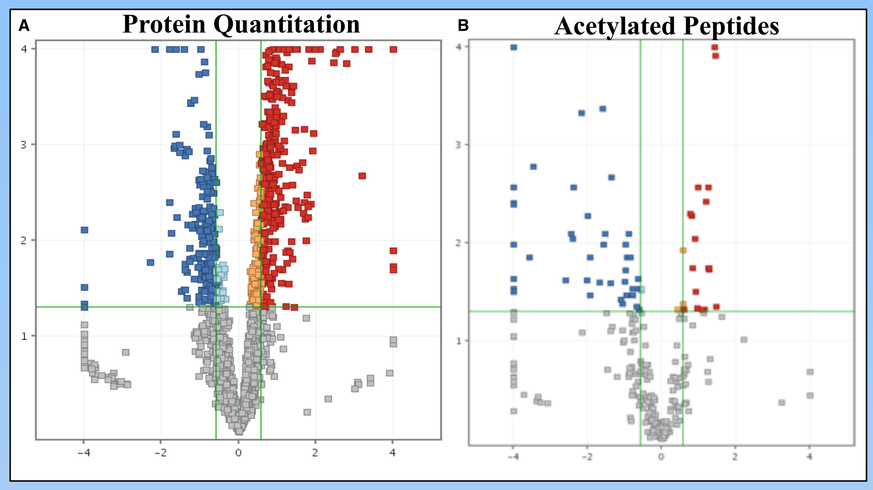


Figure 2. Benjamini-Hochburg volcano plot for (A) total proteomic analysis and (B) acetylomic analysis. (Blue = down-regulated, red = up-regulated, gray = no change; p-value <0.05)

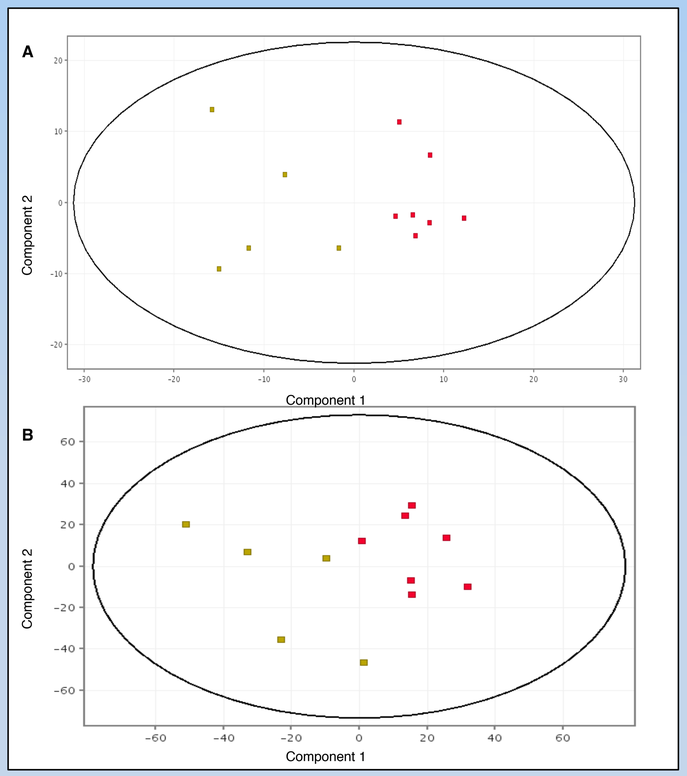


Figure 3. Principle component analysis (PCA) plot for (A) total proteomic analysis and (B) acetylomic analysis. (Blue = diabetic samples, n = 5; red = non-diabetic control samples, n = 7)

Biological Pathway	Count	%	P-value	Benjamini P-value
tricarboxylic acid cycle	12	10.3	2.20E-17	1.40E-14
mitochondrial ATP synthesis coupled proton t	10	8.6	5.10E-15	1.60E-12
metabolic process	14	12.1	9.10E-11	1.90E-08
fatty acid beta-oxidation	9	7.8	4.40E-10	7.10E-08
ATP biosynthetic process	8	6.9	6.70E-10	8.60E-08
ATP synthesis coupled proton transport	7	6	5.30E-09	5.60E-07
oxidation-reduction process	19	16.4	7.10E-08	6.50E-06
mitochondrial electron transport, NADH to ub	7	6	8.50E-07	6.80E-05
branched-chain amino acid catabolic process	5	4.3	6.70E-06	4.80E-04
mitochondrial electron transport, cytochrome	5	4.3	8.40E-06	5.40E-04
generation of precursor metabolites and ener	6	5.2	2.70E-05	1.60E-03
succinate metabolic process	4	3.4	3.40E-05	1.80E-03
protein homotetramerization	6	5.2	4.90E-05	2.40E-03
hydrogen ion transmembrane transport	6	5.2	5.30E-05	2.40E-03
aerobic respiration	5	4.3	6.60E-05	2.80E-03
oxidative phosphorylation	4	3.4	7.90E-05	3.20E-03
mitochondrial electron transport, ubiquinol to	4	3.4	1.20E-04	4.70E-03

Biological Pathway	Count	%	P-value	Benjamini P-value
SRP-dependent cotranslational protein targe	29	20.1	2.30E-36	2.20E-33
nuclear-transcribed mRNA catabolic process	30	20.8	8.50E-35	4.20E-32
viral transcription	29	20.1	5.90E-34	2.00E-31
translational initiation	30	20.8	7.50E-33	1.90E-30
rRNA processing	29	20.1	1.40E-25	2.70E-23
translation	30	20.8	8.30E-25	1.40E-22
cytoplasmic translation	6	4.2	1.90E-06	2.60E-04
extracellular matrix organization	11	7.6	6.50E-06	8.10E-04
mRNA splicing, via spliceosome	11	7.6	1.90E-05	2.10E-03

Figure 4. DAVID biological pathway enrichment for total proteomic analysis; (top) pathways significantly down-regulated, and (bottom) pathways significantly up-regulated in diabetic human samples as compared to non-diabetic control samples (cut off BF factor < 0.01)

Biological Pathway	Count	%	P-value	Benjamini P-value
branched-chain amino acid catabolic process	5	13.2	7.50E-08	2.10E-05
tricarboxylic acid cycle	5	13.2	4.60E-07	6.40E-05
metabolic process	6	15.8	3.20E-05	3.00E-03
carboxylic acid metabolic process	3	7.9	3.60E-04	2.60E-02
mitochondrial ATP synthesis coupled proton transport	3	7.9	9.70E-04	4.30E-02
ATP synthesis coupled proton transport	3	7.9	1.10E-03	4.30E-02
pyruvate metabolic process	3	7.9	1.10E-03	4.30E-02
glyoxylate metabolic process	3	7.9	1.50E-03	5.20E-02
ATP biosynthetic process	3	7.9	1.80E-03	5.80E-02
glycolytic process	3	7.9	2.50E-03	6.90E-02
response to starvation	3	7.9	2.70E-03	6.90E-02
fatty acid beta-oxidation	3	7.9	4.20E-03	9.90E-02
substantia nigra development	3	7.9	5.20E-03	1.10E-01
response to hydrogen peroxide	3	7.9	5.60E-03	1.10E-01
generation of precursor metabolites and energy	3	7.9	6.10E-03	1.10E-01
adenine transport	2	5.3	8.80E-03	1.50E-01
oxidation-reduction process	6	15.8	9.20E-03	1.50E-01
cellular oxidant detoxification	3	7.9	1.00E-02	1.50E-01

Biological Pathway	Count	%	P-value	Benjamini P-value
gluconeogenesis	2	20	2.30E-02	1.00E+00
leukocyte migration	2	20	6.40E-02	1.00E+00
protein folding	2	20	9.20E-02	1.00E+00

Figure 5. DAVID biological pathway enrichment for acetylomic analysis; (top) pathways significantly down-regulated, and (bottom) pathways significantly up-regulated in diabetic human samples as compared to non-diabetic control samples (cut off p-value < 0.01)

Conclusion

- A total of 840 proteins were identified in human kidney tissue samples based on proteomic analysis and 289 proteins from acetylomic analysis.
- Diabetic and non-diabetic samples demonstrate clear differences in protein abundance based on PCA scatter plots.
- Metabolic pathways were significantly down-regulated in diabetic cases as compared to non-diabetic controls based on total proteomic and acetylomic analyses.
- Extracellular matrix remodeling processes, including the matrix metalloproteinase matrilysin, are significantly up-regulated in diabetic samples, a hallmark of diabetic nephropathy pathogenesis.
- Future investigation will include detailed and specific analysis of proteomic and acetylomic targets significantly related to DN.

Acknowledgment

This study was supported, in part, by NIH-EY028836 (RHN). The authors thank the Mass Spectrometry Core in the Skaggs School of Pharmacy and Pharmaceutical Sciences.

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