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, podocyte injury, progression of diabetic kidney disease (CKD), 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.

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 Johns Hopkins University. Each case had three tubes containing three 10 μm curls. Tissues were deparaffinized at room temperature using xylene. 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. RCI/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 -80°C in 5% ACN + 0.1% TFA until prepared via lyophilization for total peptide and acetylome 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 166 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 acetylation analysis. 76 were significantly down-regulated and 29 were significant up-regulated (p-value < 0.05).

Figure 2. Benjamini-Hochberg volcano plot for (A) total proteomic analysis and (B) acetylated 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) acetylated analysis. (Blue = diabetic samples, n = 5; red = non-diabetic control samples, n = 7).

Biological Pathway | Count | P-value | Benjamini P-value |
---|---|---|---|
Protein Quantitation | | | |
Glucose transport | 53 | 1.20E-32 | 4.20E-32 |
Metabolic process | 85 | 7.90E-04 | 7.90E-04 |
Mitochondrial electron transport | 73 | 5.20E-02 | 5.20E-02 |
Glutathione redox process | 58 | 1.20E-02 | 1.20E-02 |
Protein ubiquitination | 36 | 3.70E-01 | 3.70E-01 |
Acetylation | 29 | 1.20E-01 | 1.20E-01 |
Acetyltransferase activity | 23 | 6.90E-01 | 6.90E-01 |
Nuclear transcription factor activity | 19 | 7.90E-01 | 7.90E-01 |
Histone acetyltransferase activity | 18 | 7.90E-01 | 7.90E-01 |
Acetyl-CoA synthetase activity | 16 | 7.90E-01 | 7.90E-01 |
Detoxification | 16 | 7.90E-01 | 7.90E-01 |
Acetyl-CoA dehydrogenase activity | 13 | 7.90E-01 | 7.90E-01 |
Acetylation | 13 | 7.90E-01 | 7.90E-01 |
Acetyl-CoA synthetase activity | 12 | 7.90E-01 | 7.90E-01 |
Detoxification | 12 | 7.90E-01 | 7.90E-01 |
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 |
---|---|---|---|
Secretory cationic protein | 25 | 1.20E-02 | 1.20E-02 |
Amino acid biosynthesis | 23 | 1.20E-02 | 1.20E-02 |
Amino acid biosynthesis | 23 | 1.20E-02 | 1.20E-02 |
Protein ubiquitination | 20 | 1.20E-02 | 1.20E-02 |
Nuclear transcription factor activity | 20 | 1.20E-02 | 1.20E-02 |
Acetyltransferase activity | 20 | 1.20E-02 | 1.20E-02 |
Acetyl-CoA synthetase activity | 19 | 1.20E-02 | 1.20E-02 |
Mitochondrial electron transport | 19 | 1.20E-02 | 1.20E-02 |
Detoxification | 19 | 1.20E-02 | 1.20E-02 |
Acetyl-CoA dehydrogenase activity | 18 | 1.20E-02 | 1.20E-02 |
Acetylation | 18 | 1.20E-02 | 1.20E-02 |
Detoxification | 18 | 1.20E-02 | 1.20E-02 |
Figure 5. DAVID biological pathway enrichment for acetylated 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).

Conclusion

- A total of 840 proteins were identified in human kidney tissue samples based on proteomic analysis and 289 proteins from acetylome 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 acetylome analyses.
- Extracellular matrix remodeling processes, including the matrix metalloproteinase matrixins, significantly up-regulated in diabetic samples, a hallmark of diabetic nephropathy pathogenesis.
- Future investigation will include detailed and specific analysis of proteomic and acetylome targets significantly related to DN.

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References
