CHARACTERIZATION OF THE MITOCHONDRIAL PROTEOME
IN PYRUVATE DEHYDROGENASE KINASE 4
WILD-TYPE AND KNOCKOUT MICE

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Master’s Thesis Committee

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DEDICATION

This work is dedicated to my wonderful family. To my best friend and husband, Kris, thank you for your love, encouragement, support, and devotion. To my beautiful daughters, Maliah and Halle, you are my pride and joy. To my parents, Angie and David, thank you for supporting me in achieving such great success throughout my education. To my grandparents, Mary and Bud, I am so grateful for your endless support and help. To my mother-in-law, brother, sister, aunts, and cousins, I truly appreciate each of you for caring for my children and assisting every way possible during this process.
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ABSTRACT
Heather Nicole Ringham

CHARACTERIZATION OF THE MITOCHONDRIAL PROTEOME IN PYRUVATE DEHYDROGENASE KINASE 4 WILD-TYPE AND KNOCKOUT MICE

The goal of this study was to determine the effect of a PDK4 (pyruvate dehydrogenase kinase isoenzyme 4) knock-out on mitochondrial protein expression. A 2-D gel based mass spectrometry approach was used to analyze the mitochondrial proteomes of PDK4 wild-type and knockout mice. Mitochondria were isolated from the kidneys of mice in both well-fed and starved states. Previous studies show PDK4 increases greatly in the kidney in response to starvation and diabetes suggesting its significance in glucose homeostasis. The mitochondrial fractions of the four experimental groups (PDK4+/+ fed, PDK4+/+ starved, PDK4−/+ fed, and PDK4−/+ starved) were separated via large-format, high resolution two-dimensional gel electrophoresis. Gels were scanned, image analyzed, and ANOVA performed followed by a pair-wise multiple comparison procedure (Holm-Sidak method) for statistical analysis. The abundance of a total of 87 unique protein spots was deemed significantly different (p<0.05). 22 spots were up- or down-regulated in the fed knockout vs. fed wild-type; 26 spots in the starved knockout vs. starved wild-type; 61 spots in the fed vs. starved wild-types; and 44 in the fed vs. starved knockouts; 63 spots in the PDK4+/+ fed vs. PDK4−/+ starved; and 42 spots in the PDK4−/+ fed vs. PDK4−/+ starved. Altered protein spots were excised from the gel, trypsinized, and identified via tandem mass spectrometry (LC-MS/MS). Differentially expressed proteins identified with high confidence include ATP synthase proteins, fatty acid
metabolism proteins, and components of the citric acid cycle and electron transport chain. Proteins of interest were analyzed with Ingenuity Pathway Analysis (IPA) to examine relationships among the proteins and analyze biological pathways, as well as ontological analysis with Generic Gene Ontology (GO) Term Mapper. IPA found a number of canonical pathways, biological functions, and functional networks associated with the 87 proteins. Oxidative phosphorylation was the pathway associated with a majority of the proteins, while the largest network of proteins involved carbohydrate metabolism and energy production. Overall, the effects of starvation were more extensive on mitochondrial protein expression than the PDK4 knockout.
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<tr>
<td>2DE</td>
<td>Two-Dimensional Electrophoresis</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>ALA</td>
<td>D-aminolevulinic acid</td>
</tr>
<tr>
<td>ALAS</td>
<td>D-aminolevulinic acid synthase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
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<td>CAC</td>
<td>Citric Acid Cycle</td>
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<tr>
<td>CHAPS</td>
<td>3-[(3-cholamidopropyl) dimethyl/ammonio]-l-propane-sulfonate</td>
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</tr>
<tr>
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<tr>
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<td>Dithiothreitol</td>
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<td>Erv1</td>
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<tr>
<td>ETC</td>
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<td>ETF</td>
<td>Electron Transfer Flavoprotein</td>
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<tr>
<td>FA</td>
<td>Formic Acid</td>
</tr>
<tr>
<td>FADH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Flavin Adenine Dinucleotide</td>
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<tr>
<td>GO</td>
<td>Gene Ontology</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat Shock Protein</td>
</tr>
<tr>
<td>IEF</td>
<td>Isoelectric Focusing</td>
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<tr>
<td>IPA</td>
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<td>IPG</td>
<td>Immobilized pH Gradient</td>
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<td>IPI</td>
<td>International Protein Index</td>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodaltons</td>
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<tr>
<td>Mia40</td>
<td>Mitochondrial intermembrane space import &amp; assembly protein 40</td>
</tr>
<tr>
<td>mM</td>
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<tr>
<td>O₂</td>
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<tr>
<td>PDC</td>
<td>Pyruvate Dehydrogenase Complex</td>
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<td>PDK4</td>
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<tr>
<td>pI</td>
<td>Isoelectric Point</td>
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<tr>
<td>PPM</td>
<td>Parts Per Million</td>
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<tr>
<td>Q</td>
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<td>QH₂</td>
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<td>Trans-Proteomic Pipeline</td>
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<tr>
<td>µm</td>
<td>Micrometer</td>
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<tr>
<td>Vh</td>
<td>Volt-hours</td>
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