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

Heather Nicole Ringham

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Frank A. Witzmann, Ph.D., Chair

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Robert A. Harris, Ph.D.

Master's Thesis Committee

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Mu Wang, Ph.D.

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.

Frank A. Witzmann, Ph.D., Chair

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ABBREVIATIONS

2DE	Two-Dimensional Electrophoresis
ACN	Acetonitrile
ALA	D-aminolevulinic acid
ALAS	D-aminolevulinic acid synthase
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
CAC	Citric Acid Cycle
CHAPS	3-[(3-cholamidopropyl) dimethyl/ammonio]-l-propane-sulfonate
CoA	Coenzyme A
CoQ	Coenzyme Q
DTT	Dithiothreitol
Erv1	Essential for respiration and vegetative growth 1
ETC	Electron Transport Chain
ETF	Electron Transfer Flavoprotein
FA	Formic Acid
$FADH_2$	Flavin Adenine Dinucleotide
GO	Gene Ontology
HSP	Heat Shock Protein
IEF	Isoelectric Focusing
IPA	Ingenuity Pathway Analysis
IPG	Immobilized pH Gradient
IPI	International Protein Index

kDa	Kilodaltons
Mia40	Mitochondrial intermembrane space import & assembly protein 40
mM	Millimolar
MW	Molecular Weight
NADH	Nicotinamide Adenine Dinucleotide
NSI	Nanospray Ionization
O_2	Molecular Oxgyen
PDC	Pyruvate Dehydrogenase Complex
PDK2	Pyruvate Dehydrogenase Kinase, isoenzyme 2
PDK4	Pyruvate Dehydrogenase Kinase, isoenzyme 4
$PDK4^{+/+}$	Pyruvate Dehydrogenase Kinase, isoenzyme 4, wild-type
PDK $4^{-/-}$	Pyruvate Dehydrogenase Kinase, isoenzyme 4, knockout
pI	Isoelectric Point
PPM	Parts Per Million
Q	Ubiquinone
QH_2	Ubiquinol
SCAD	Short-Chain specific Acyl-CoA Dehydrogenase
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TOM	Translocase of Outer Membrane
Tim9-Tim10	Translocase of the inner membrane 9-10
TPP	Trans-Proteomic Pipeline
µm	Micrometer
Vh	Volt-hours