STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Regulation of cardiometabolic activity through a Citric Acid Cycle metabolon

Background: Cyclophilin D (CypD) not only regulates the mitochondrial permeability transition pore (mPTP) in development, but also the assembly of the electron transport chain (ETC) into respirasomes. Knockout (KO) of CypD in mouse hearts leads to earlier ETC activity and a more mature phenotype. The substrates NADH and $FADH_2$ are generated by the tricarboxylic acid (TCA) cycle. Whether CypD affects the activity of the TCA cycle has not been investigated. It is also not known how CypD, or its regulation by acetylation (AcCypD), affects the organization of the TCA enzymes into functional super complexes called metabolons.

Objective: Our objective is to determine how differential expression of CypD affects isocitrate dehydrogenase (IDH, a TCA cycle enzyme) activity in the presence of Ca^{2+}, ADP, and CsA and investigate the existence of a TCA cycle metabolon that aids in cellular respiration.

Methods: Our methods included isolating mitochondria by differential centrifugation, native gel electrophoresis, in-gel assays, and Western blotting. We measured the activity of isocitrate dehydrogenase (IDH) using conditions that favor (Ca^{2+}) or inhibit (ADP and cyclosporin A (CsA)) mPTP opening. TCA cycle metabolons were visualized using native gels by in-gel assays (IGA) and Western blotting.

Hypothesis: We hypothesize that CypD regulates the TCA cycle enzymes which interact to form a metabolon.

Results: The activity of IDH in heart mitochondria from WT mice, but not from CypD KO and AcCypD mice, increased in the presence of Ca^{2+}, while neither CsA nor ADP had an effect. In-gel assays showed that in the heart, IDH and other TCA cycle enzymes (citrate synthase, aconitase, fumarase, and malate dehydrogenase) are associated in metabolons greater than 690 kDa. Western blotting showed the metabolon has the same molecular weight as complex I. This indicates that these enzymes of the TCA cycle may bind to complex I or the metabolon has a similar molecular weight as complex I. In-gel assays of liver mitochondria showed less signal for high molecular weight metabolons, with large signals at less than 440 kD. We did not observe any specific differences in CypD KO and AcCypD samples.

Conclusion: There is evidence for a TCA cycle metabolon in mouse hearts, which could optimize the channeling of NADH and $FADH_2$ from the TCA cycle into the ETC.