

STRONG CHILDREN'S RESEARCH CENTER

Summer 2014 Research Scholar

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ABSTRACT

Title:

Mutations in Cyclophilin-D, Mitochondrial Maturation, and Cardiomyocyte Differentiation in Neonatal Mice

Background:

Cyclophilin-D (Cyp-D) is a 20 kDa mitochondrial matrix chaperone protein that coordinates protein folding and regulates the function of ATP synthase and the mitochondrial Permeability Transition Pore (mPTP) (Figure 1). ATP synthase (Complex V of the Electron Transport Chain) is a 660 kDa multi-protein complex that consists of two major components, F₀ and F₁, and synthesizes ATP (Figure 1). The mPTP is a large channel in the inner mitochondrial membrane that, when opened under pathologic conditions, causes mitochondrial dysfunction and cell death. The Porter lab recently showed that the c-ring of F₀ forms the mPTP (Alavian et al. 2014) (Figure 1).

The Porter lab has shown that despite the rapid cell death that is caused by mPTP opening, the mPTP is open during the early embryonic period and does not induce cell death. The pore eventually closes after embryonic day 9.5 in mice (which corresponds to E22 in the human embryo) to promote maturation of the mitochondria (Hom 2011). The mechanism of this event is not yet known, but the Porter lab's data suggest that Cyp-D plays a major role. The Porter lab seeks to understand how and where Cyp-D regulates ATP synthase and mPTP assembly and how acetylation of Cyp-D may promote the efficient production of cellular energy.

The greatest early embryonic consumer of energy is the heart, which grows from approximately 0.025 mm³ to 0.85 mm³ between E8.5 and E14.5 (Soufer et al. 2003) (Figure 2). In order to grow at this exertive rate, the heart requires intact mitochondria with a closed mPTP to optimize ATP synthesis. Immature mitochondria plagued by persistent permeability transition may cause a variety of acute and chronic disorders including cardiomyopathies, congenital heart disease, and heart failure. Thus, a deeper understanding of the mPTP and Cyp-D may direct e subunit targeted therapies to prompt mitochondrial maturation in failing, immature hearts.

Objective:

In order to study the effects of modifying Cyp-D function, we introduced four separate single base pair mutations that caused amino acid mutations to Cyp-D via site directed mutagenesis (Figure 3, 4). Our main objective is to study how these mutations affect mitochondrial differentiation in neonatal mice cardiomyocytes. The objective of this summer project was to create the mutated reagents and optimize conditions that would allow us to accomplish the main objective.

Results:

Summary:

The sequencing indicated the presence of the four desired mutations (Figure 4).

The nuclei, mitochondria, and α -actinin were successfully labeled by DAPI, GFP, and RFP, respectively (Figures 5,6).

There were very few cardiomyocytes recovered in these cultures.

Cardiomyocytes were poorly transfected but were visible and marked by ~~one~~ contractile apparatus ~~Z~~ bands (Figure 5).

There was poor transfection efficiency although manipulation of the conditions increased this efficiency in fibroblasts.

We observed great variation in the mitochondrial networks of ~~cardiomyocytes~~ transfected cells, even within a single treatment (Figure 5).

