Validation of a Novel Mouse Model of Cox6a2 Knockdown

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Abstract

Chronic limb threatening ischemia (CLTI) is characterized by ischemic pain at rest, tissue necrosis, and gangrene. There are currently no effective treatment options for CLTI patients, despite its association with high mortality rates and limb amputation. We identified a unique mitochondrialopathy in CLTI patient limb skeletal muscles that was recapitulated in a preclinical mouse model of PAD, hindlimb ischemia (HLI). We further identified reductions in cytochrome oxidase 6a2 (Cox6a2), a regulatory protein subunit of cytochrome c oxidase (complex IV of the mitochondria) in the limb skeletal muscle of mitochondria of both CLTI patients and BALB/c mice. Cox6a2 is expressed only in mature, striated muscle (skeletal and cardiac) and is a potential genetic modifier of tissue loss in HLI. This project was designed to: 1) validate a novel and inducible model of skeletal muscle specific Cox6a2 knockdown in ischemia resistant C57BL/6J mice, and 2) determine whether Cox6a2 was required for muscle survival and regeneration after the onset of HLI. After following a prescribed breeding scheme, genotyping confirmed the creation of the desired HSA-MCM;Cox6a2fl/fl mice. Subsequent experiments established the validity of skeletal muscle specific complex IV deficiency as central to the ischemic mitochondrialopathy and myopathy that occurs in the peripheral limb of mice. Together, this data suggests that skeletal muscle Cox6a2 is a new target for therapeutic intervention.

Research Hypothesis

Cox6a2 loss results in greater susceptibility to ischemic muscle myopathy, creating a local muscle environment insufficient to support the vasculature.

Fig 1. Breeding Scheme

Fig 2. Validation of Inducible HSA-MCM:Cox6a2fl/fl Mice

A. WT

B. HOMO

Fig 3. Skeletal Muscle Morphology and Function

A. WT

B. Cox6a2-5ScR1 homozygous

C. Cox6a2 Ex1 homozygous

D. Cox6a2 fl/fl

E. Cox6a2 fl/+

Fig 3. Restoration of Paw Perfusion

Fig 4. Restoration of Peripheral Blood Flow was Determined Using LDPI. (A) Representative laser Doppler perfusion images (LDPI) of the ischemic left (Ish. (L)) and control right (Ctrl. (R)) plantar paws in the prone position prior to HLI (pre), immediately post-HLI (post), and at all other following HLI. (B) Quantification of flux measurements pre-post, and at 0t represented as a ratio of the left/right limbs.

Summary of Findings

- The described breeding scheme was effective in generating HSA-MCM;Cox6a2fl/fl mice, which was verified through genotyping.
- Cox6a2 loss in ischemia-resistant C57BL/6J mice creates a muscle that is more susceptible to ischemic injury.
- The phenotype of these mice is marked by functional deficits (maximum force production) and muscle damage (lesion area).

References