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Long term rapamycin treatment improves mitochondrial DNA quality in aging mice

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ABSTRACT

Age-induced mitochondrial DNA deletion mutations may underlie cell loss and tissue aging. Rapamycin extends mouse lifespan and modulates mitochondrial quality control. We hypothesized that reduced deletion mutation abundance may contribute to rapamycin's life extension effects. To test this hypothesis, genetically heterogeneous male and female mice were treated with rapamycin, compounded in chow at 14 or 42 ppm, from 9 months to 22 months of age. Mice under a 40% dietary restriction were included as a control known to protect mtDNA quality. To determine if chronic rapamycin treatment affects mitochondrial DNA quality, we assayed mtDNA deletion frequency and electron transport chain deficient fiber abundances in mouse quadriceps muscle.

At 42 ppm rapamycin, we observed a 57% decrease in deletion frequency, a 2.8-fold decrease in ETC deficient fibers, and a 3.4-fold increase in the number of mice without electron transport chain deficient fibers. We observed a similar trend with the 14 ppm dose. DR significantly decreased ETC deficient fiber abundances with a trend toward lower mtDNA deletion frequency. The effects of rapamycin treatment on mitochondrial DNA quality were greatest in females at the highest dose. Rapamycin treatment at 14 ppm did not affect muscle mass or function. Dietary restriction also reduced deletion frequency and ETC deficient fibers. These data support the concept that the lifespan extending effects of rapamycin treatment result from enhanced mitochondrial DNA quality.

1. Introduction

Mitochondrial dysfunction contributes to mammalian aging.(Sun et al., 2016) With age, somatically derived mtDNA deletions clonally accumulate within a subset of individual cells. These deletion mutations are large, centered in the mitochondrial major arc, and disrupt multiple genes that encode the protein subunits necessary for oxidative phosphorylation. For example, a previously described murine major arc deletion ablates subunit 3 of cytochrome *c* oxidase, four subunits of NADH dehydrogenase, and five transfer RNAs·(Brossas et al., 1994; Tanhauser and Laipis, 1995; Taylor et al., 2014) When the intracellular deletion abundance exceeds 90%, the electron transport chain (ETC) function is disrupted.(Herbst et al., 2007) These cells lack cytochrome *c* oxidase (COX) activity. COX negative, ETC deficient cells, have been detected in the brain, heart, kidney, and skeletal muscle of aged mammals and in the mitochondrial mutator mice.(Wanagat et al., 2001; Ekstrand et al., 2007; McKiernan et al., 2007; Baris et al., 2015).

In skeletal muscle, electron transport chain deficiency is localized to muscle fiber segments where the deletion mutations have accumulated to high levels (Fig. 1).(Herbst et al., 2007; Lushaj et al., 2008) ETC deficient regions atrophy, split, and undergo cell death and contribute to the age-induced loss of skeletal muscle fibers.(Wanagat et al., 2001; Cheema et al., 2015) In humans, quadriceps muscle fiber number decreases by ~40% between 50 and 80 years of age.(Lexell et al., 1988) In rodents, fiber loss, mtDNA deletions, and ETC deficient fibers are retarded by dietary restriction (Bua et al., 2004), but the mechanisms are unclear and this intervention has not seen broad clinical application.

Like dietary restriction, rapamycin treatment broadly decelerates age-related pathologies and extends lifespan in mammals.(Wilkinson et al., 2012) Rapamycin inhibits mTOR, which directly controls

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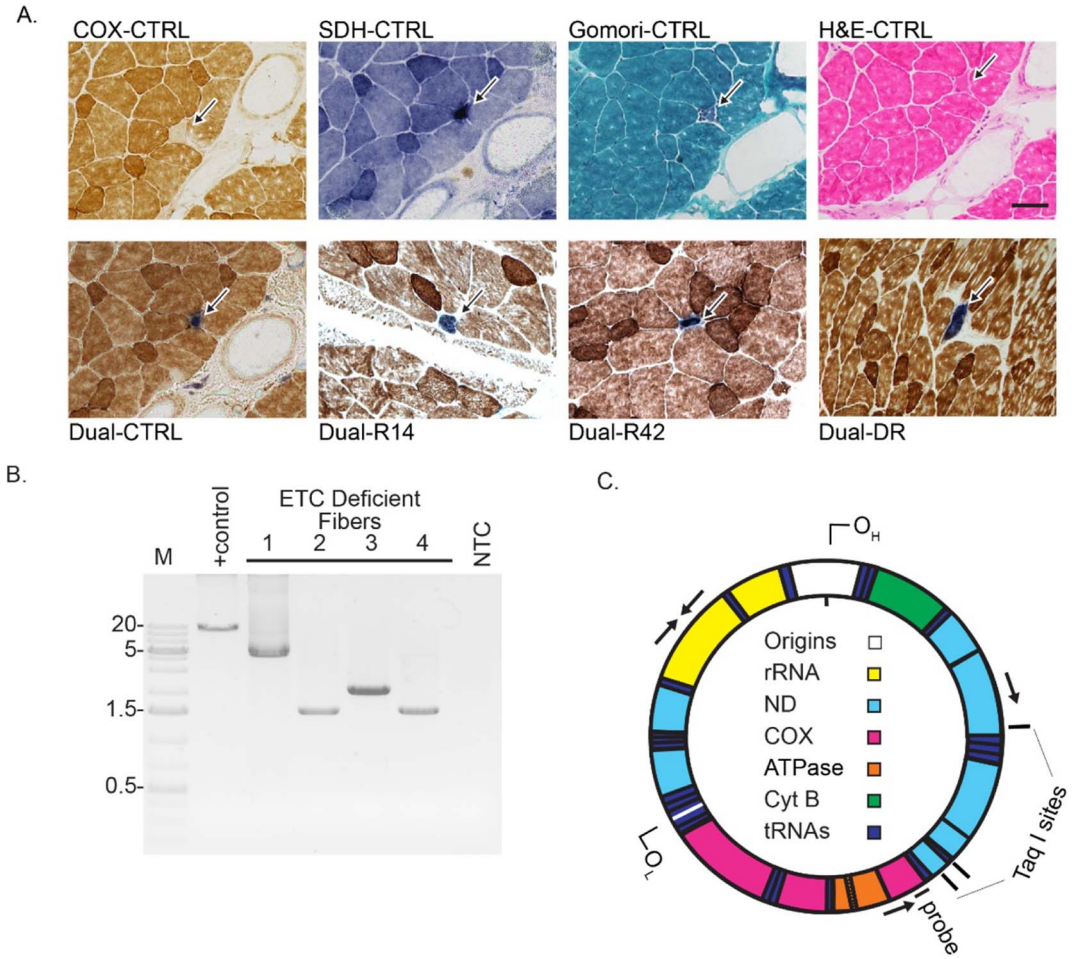

Figure 1. A. Electron transport chain deficient fibers from 22-month old mice. Top micrographs: Serial skeletal muscle histological sections from a control mouse. A single ETC deficient fiber is denoted by the black arrow. Bottom micrographs: Dual COX and SDH staining was used to identify ETC deficient fibers (black arrow) from control, 14 ppm rapamycin (R14), 42 ppm rapamycin (R42), and dietary restricted (DR) mice. The black bar indicates 100 μm. B. Detection of mtDNA deletion mutations in laser microdissected ETC deficient fibers. C. Primer and Taq I restriction site locations used to detect deletions in the major arc of mouse mitochondrial DNA.

autophagy and leads to selective degradation of organelles including dysfunctional mitochondria.(Twig et al., 2008) Activation of mitophagy has been suggested to control mtDNA quality through elimination of dysfunctional mitochondria.(de Grey, 1997; Lemasters, 2005; Suen et al., 2010) In mtDNA mutation containing cybrid cells, rapamycin treatment robustly induced mitophagy (Gilkerson et al., 2012) and decreased *in vitro* mitochondrial mutation frequency (Dai et al., 2014). In a *Drosophila* model of mtDNA heteroplasmy, rapamycin treatment decreased the abundance of deletion bearing mtDNA and improved mtDNA quality.(Kandul et al., 2016) In a mitochondrial helicase mutant mouse model, rapamycin treatment for 70 days ameliorated myopathic progression. (Khan et al., 2017) The *in vivo* effects of rapamycin on age-induced mammalian mtDNA deletions or ETC deficient cells are unknown.

We hypothesized that long term rapamycin treatment in mice would reduce mtDNA deletion mutation frequency and ETC deficient fiber abundance. To test this hypothesis, we measured mtDNA deletion abundances (Brossas et al., 1994; Tanhauser and Laipis, 1995; Taylor et al., 2014) and ETC deficient fibers in 22 month old mice treated with rapamycin for 15 months (Drake et al., 2013; Miller et al., 2014). We found that rapamycin treatment decreased deletion frequency and ETC deficient fiber abundance.

2. Methods

2.1. Mice and experimental treatments

The mice used in this study were experimentally manipulated as part of the National Institute on Aging Intervention Testing Program. Details of breeding and husbandry of the genetically heterogenous UM-HET3 mice and experimental treatments with rapamycin have been previously described.(Drake et al., 2013, Miller et al., 2014) Briefly, at 9 months of age, mice were fed a diet containing encapsulated rapamycin at 14 or 42 ppm (mg of drug per kg of food). We denote these as R14 or R42, respectively. Other mice were placed on a 40% dietary restriction (DR) diet. Muscle samples from DR mice were included as controls as DR decreased muscle ETC deficient fibers in rats.(Bua et al., 2004) Predetermined cross-sectional cohorts were euthanized at 12 or 22 months following physiological measurements. At 22-months of age, in this strain, represents a population from which only about 5% of females to 20% of males have died spontaneously, minimizing selection bias effects.

2.2. Muscle physiological measurements

Mouse muscle physiological measurements including maximal isometric force (mN) and maximal specific force (N/cm^2) were done as previously described.(Sataranatarajan et al., 2015) *In situ* gastrocnemius muscle contractile properties were measured, as described by

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