Experimental Gerontology 80 (2016) 36–42

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Comparative gene expression and phenotype analyses of skeletal muscle from aged wild-type and PAPP-A-deficient mice

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ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form 5 April 2016
Accepted 6 April 2016
Available online 13 April 2016

Section Editor: Christiaan Leeuwenburgh

Keywords:
Pregnancy-associated plasma protein-A
Soleus
Mitochondria

ABSTRACT

Mice deficient in pregnancy-associated plasma protein-A (PAPP-A) have extended lifespan associated with decreased incidence and severity of degenerative diseases of age, such as cardiomyopathy and nephropathy. In this study, the effect of PAPP-A deficiency on aging skeletal muscle was investigated. Whole-genome expression profiling was performed on soleus muscles from 18-month-old wild-type (WT) and PAPP-A knock-out (KO) mice of the same sex and from the same litter ('womb-mates') to identify potential mechanisms of skeletal muscle aging and its retardation in PAPP-A deficiency. Top genes regulated in PAPP-A KO compared to WT muscle were associated with increased muscle function, increased metabolism, in particular lipid metabolism, and decreased stress. Fiber cross-sectional area was significantly increased in solei from PAPP-A KO mice. In vitro contractility experiments indicated increased specific force and decreased fatigue in solei from PAPP-A KO mice. Intrinsic mitochondrial oxidative capacity was significantly increased in skeletal muscle of aged PAPP-A KO compared to WT mice. Moreover, 18-month-old PAPP-A KO mice exhibited significantly enhanced endurance running on a treadmill. Thus, PAPP-A deficiency in mice is associated with indices of healthy skeletal muscle function with age.

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1. Introduction

Aging is associated with loss of skeletal muscle mass and compromised function with attendant frailty and increased risk of incapacitating injuries (Marzetti et al., 2009; Rosenberg, 1997). This predisposition to disability and mortality in our ever-growing elderly population is a significant public health issue. A better understanding of the factors involved in this age-related decrease in muscle function could suggest novel approaches to prevent or delay the process.

Lee et al. (1999) used a high-density oligonucleotide array to analyze the genomic profile of the aging process in mouse skeletal muscle, and found a gene expression pattern indicative of a marked stress response and lowered expression of metabolic and biosynthetic genes in old versus young adult mice. Moreover, these changes in gene expression were prevented by caloric restriction, which is known to increase healthy lifespan (Minor et al., 2010). Similarly, Park and Prolla (2005) studied gene expression profiles of aging cardiac and skeletal muscles in mice. The transcriptional pattern suggested that aging increases heat shock response and genes induced by oxidative stress, toxins, and DNA damage, and decreases those involved with energy metabolism. Caloric restriction resulted in a transcriptional shift toward energy metabolism, in particular lipid metabolism, and decreases in mRNA encoding inducible genes involved in metabolic detoxification, oxidative stress, and protein misfolding. Lanza et al. (2012) used whole-genome expression profiling and proteomics to determine the effect of age and caloric restriction on mouse skeletal muscle. In that study, aging was associated with gene expression patterns consistent with up-regulation of pyruvate metabolism, oxidative phosphorylation, and cellular response to oxidative stress. Caloric restriction attenuated or reversed these changes. Thus, several gene expression profile studies using DNA microarrays suggested that skeletal muscle aging is associated with increased stress response genes and diminished energy metabolism. Caloric restriction could prevent or reverse these changes and, thus, preserve muscle function in aging. However, long-term caloric restriction in humans is not a practical approach to the problem of muscle aging.

Pregnancy-associated plasma protein-A (PAPP-A) is a novel zinc metalloproteinase that enhances local insulin-like growth factor (IGF) action through cleavage of inhibitory binding proteins (Conover, 2012). Diminished IGF action is associated with increased lifespan in a variety of species, and may contribute to the extended lifespan with caloric restriction (Barbieri et al., 2003; Berryman et al., 2008). PAPP-A knock-out (KO) mice live 30–40% longer than their wild-type littermates, with decreased incidence and severity of degenerative diseases of age, such as nephropathy and cardiomyopathy (Conover et al., 2010). However, skeletal muscle of PAPP-A KO mice has not

Abbreviations: PAPP-A, pregnancy-associated plasma protein-A; WT, wild-type; KO, knock-out; IGF, insulin-like growth factor; CSA, cross-sectional area; mtDNA, mitochondrial DNA.

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http://dx.doi.org/10.1016/j.exger.2016.04.005
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been examined in any detail. In this study we compared genome-wide expression profiles in skeletal muscle of aged PAPP-A KO and wild-type mice that were matched for sex, intrauterine environment and postnatal housing. Furthermore, we employed several methodologies to evaluate skeletal muscle phenotype and function in aged PAPP-A KO and wild-type mice.

2. Experimental procedures

2.1. Mice

All animal procedures followed protocols reviewed and approved by the Institutional Animal Care and Use Committee of Mayo Clinic. Mice heterozygous for PAPP-A gene deletion were crossbred (14 breeder cages) as described previously (Conover et al., 2004). Both male and female offspring were used in experiments, with approximately equal distribution.

2.2. Womb-mate tissue

"Womb-mate" status was assigned to those pups that were from the same litter, of the same sex and paired PAPP-A KO and wild-type (WT). This was to minimize confounding variables of intrauterine development and sex. Womb-mate pairs were then housed together for 18 months to minimize potential confounding variables of environment. At this time tissues were harvested and frozen at —80 °C. For this study, we focused on the soleus muscle, which is composed of predominantly oxidative fiber types.

2.3. Illumina microarray protocol

RNA was extracted from solei (2 pooled from each mouse) using QIAGEN RNeasy Fibrous Tissue Kit (QIAGEN, Valencia, CA). All subsequent procedures and expression analyses were carried out by the Advanced Genomics Technology Center at Mayo Clinic. The quality of total RNA samples was assessed using the Agilent Bioanalyzer 2100 (Santa Clara, CA). Labeling of high quality samples was performed according to manufacturer's instructions for the Illumina Total Prep RNA Amplification Kit (Life Technologies, Grand Island, NY). Briefly, 200 ng of total RNA was reverse transcribed with T7 Oligo d(T) to create second strand cDNA. Subsequently, the products were column-purified and then in vitro transcribed to generate biotin-labeled cRNA. cRNA products were column-purified and hybridized onto Illumina Mouse Whole Genome 6 Beadchips for 16 h at 58 °C. Following hybridization, the arrays were washed, stained with streptavidin-cy3 conjugate, and then scanned in an Illumina BeadArray Reader. All quality assessment parameters were determined to be within normal ranges before proceeding to the final data reduction. Three womb-mate pairs were run simultaneously on a single Beadchip. Six pairs were analyzed (three males and three females).

2.4. Fiber cross-sectional area (CSA)

Tendon-to-tendon soleus muscles were isolated, fixed in 10% formalin and embedded in paraffin. Five-micrometer sections were cut from the middle of the muscle and stained with hematoxylin and eosin. Approximately 70 fiber CSAs were measured from solei from two separate mice of each genotype using Image J software.

2.5. Muscle contractility in vitro

Isometric force and fatigue were measured as previously described (Greising et al., 2011, 2013).

2.6. Citrate synthase activity

Citrate synthase (a marker for mitochondrial content) activity was measured spectrophotometrically and expressed relative to tissue wet weight (Lanza et al., 2012).

2.7. Mitochondrial DNA (mtDNA) copy number

DNA was extracted from frozen tissues using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA). Relative mtDNA copy numbers were determined by real-time PCR (Applied Biosystems 7900HT Sequence Detection System) using primer/probe sets targeted to mtDNA-encoded NADH dehydrogenase subunits 1 (ND1) and 4 (ND4), as described previously (Lanza et al., 2012). Samples were run in duplicate and normalized for the nuclear reference gene 28S ribosomal DNA.

2.8. Mitochondrial ATP production rate

Mitochondrial ATP production rate was measured in isolated mitochondria with a bioluminescent technique, as described previously (Short et al., 2005).

2.9. Treadmill

Physical function was characterized by measuring running time and distance using a motorized 6-lane treadmill (Columbus Instruments, Columbus, OH), as previously described (LeBrasseur et al., 2009). In brief, 18–20 WT and PAPP-A KO mice were acclimated to the treadmill for three consecutive days for 5 min at a speed of 10 m/min at a 5% grade. The next day, mice were run on the treadmill at an initial speed of 10 m/min and grade 5% for 5 min, and then every subsequent 2 min the speed was increased by 2 m/min until the mice were exhausted. Exhaustion was defined as the inability of the mouse to remain on the treadmill despite an electric shock stimulus and mechanical prodding for a period of 5 s. Mice were run in batches, with WT and KO mice part of each batch. These experiments were performed by experienced personnel in the Aging Animal Phenotyping Core of the Kogod Center on Aging at Mayo Clinic.

2.10. Statistical methods

Microarray analyses were conducted using log-base2 of the gene expression values, and programming was done in the R statistical language (Team, 2012). Data were normalized using the "fastlo" algorithm (Ballman et al., 2004). Pre- and post-normalization quality controls were assessed visually using minus versus average plots and box-plots. Paired *t*-tests were used to test for significant differences between the PAPP-A KO and WT mice; fold changes are reported as PAPP-A KO relative to WT. False discovery rates (FDR) were calculated using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). Complete microarray data have been submitted to the National Center for Biotechnology Informatics (NCBI) Gene Expression Omnibus (GEO) database. The accession number for this data set is pending [submission in process].

Kaplan-Meier survival curves were compared using log-rank test in JMP Pro 9.0.1. Data comparing WT and PAPP-A KO groups, presented as mean ± SEM, were analyzed by unpaired *t*-test. Significance was set at $P < 0.05$.

3. Results

3.1. Muscle gene expression

PAPP-A KO and wild-type (WT) womb-mate tissue samples provided robust statistical power for paired *t*-test analysis of differentially expressed genes. This is illustrated by examples in Fig. 1. Each of the

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