Biochemical and Biophysical Research Communications 459 (2015) 66–70

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

G7731A mutation in mouse mitochondrial *tRNA^{Lys}* regulates late-onset disorders in transmitochondrial mice

Akinori Shimizu [a], Takayuki Mito [a], Osamu Hashizume [a], Hiromichi Yonekawa [b], Kaori Ishikawa [a], Kazuto Nakada [a, c], Jun-Ichi Hayashi [a, c, d, *]

[a] Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan
[b] Department of Laboratory Animal Science, The Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo, 156-8506, Japan
[c] International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan
[d] TARA Center, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan

ARTICLE INFO

Article history:
Received 6 February 2015
Available online 23 February 2015

Keywords:
G7731A mtDNA mutation
Mitochondrial *tRNA^{Lys}* gene
Transmitochondrial mice
Respiration defects
Late-onset disorders
Mitochondrial disease models

ABSTRACT

We previously generated mito-mice-tRNA^{Lys7731} as a model for primary prevention of mitochondrial diseases. These mice harbour a G7731A mtDNA mutation in the *tRNA^{Lys}* gene, but express only muscle weakness and short body length by four months. Here, we examined the effects of their aging on metabolic and histologic features. Unlike young mito-mice-tRNA^{Lys7731}, aged mito-mice-tRNA^{Lys7731} developed muscle atrophy, renal failures, and various metabolic abnormalities, such as lactic acidosis and anemia, characteristic of patients with mitochondrial diseases. These observations provide convincing evidence that the respiration defects induced by high G7731A mtDNA levels cause these late-onset disorders that are relevant to mitochondrial diseases.

1. Introduction

The accumulation of mitochondrial DNA (mtDNA) with pathogenic mutations is proposed to be closely associated with mitochondrial diseases, normal aging, and age-associated disorders, including diabetes, because of its induction of significant respiration defects [1–3]. Three most prevalent mitochondrial diseases are chronic progressive external ophthalmoplegia (CPEO), due to mtDNA with large-scale deletions (ΔmtDNA); myoclonic epilepsy with ragged-red fibers (MERRF), due to single-point mutations in the *tRNA^{Lys}* gene; and mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS), due to single-point mutations in the *tRNA$^{Leu(UUR)}$* gene [1–3]. Subsequent studies [4–6] have proved the pathogenicities of these mtDNA mutations by inducing respiration defects after the transfer of patient-derived mutated mtDNA into mtDNA-less human cells. However, it has not been established unequivocally whether the respiration defects due to the accumulation of mtDNA with pathogenic mutations in the tissues actually induce the various clinical phenotypes observed in patients with mitochondrial diseases or with age-associated disorders.

This issue was resolved in part by our previous studies [7–11], in which we generated transmitochondrial mice (mito-mice) carrying exogenously introduced mouse mtDNA with mutations orthologous to those found in patients with mitochondrial diseases. For example, mito-mice-Δ, which harbored mouse ΔmtDNA and therefore corresponded to disease models for CPEO, simultaneously expressed respiration defects, which were induced by accumulated ΔmtDNA, and disease phenotypes corresponding to those of CPEO in humans [7,8]. Therefore, experiments in mito-mice could provide convincing evidence that the respiration defects due to mtDNA mutations regulate clinical abnormalities relevant to mitochondrial diseases or age-associated disorders.

We recently generated mito-mice-tRNA^{Lys7731}, which harbour mtDNA that contain a pathogenic G7731A mutation in the *tRNA^{Lys}* gene (G7731A mtDNA), as a model for primary prevention of mitochondrial diseases [12]. Because a G8328A mutation orthologous to the mouse G7731A mutation occurs in patients with mitochondrial diseases [13,14], mito-mice-tRNA^{Lys7731} can be used

Abbreviations: mtDNA, mitochondrial DNA; ΔmtDNA, mtDNA with large-scale deletions; CPEO, chronic progressive external ophthalmoplegia; MERRF, myoclonic epilepsy with ragged red fibers; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes syndrome; COX, cytochrome c oxidase; RRFs, ragged red fibers; SDH, succinate dehydrogenase.

* Corresponding author. TARA Center, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan. Fax: +81 298536650.
E-mail address: jih45@biol.tsukuba.ac.jp (J.-I. Hayashi).

http://dx.doi.org/10.1016/j.bbrc.2015.02.070
0006-291X/

as prospective models for diseases that, like MERRF, are caused by mutations in the mitochondrial *tRNA^{Lys}* gene. Young mito-mice-tRNA^{Lys7731} with high levels of G7731A mtDNA expressed respiration defects and the resultant phenotypic features characteristic of mitochondrial diseases, such as short body length and muscle weakness, but they did not exhibit ragged red fibers (RRFs) or other traits seen in patients with mitochondrial diseases [12].

Here, to compare the effects of aging on the expression of phenotypes characteristic of mitochondrial diseases and age-associated disorders, we used aged mito-mice-tRNA^{Lys7731} that share the same nuclear background but carry either low or high levels of G7731A mtDNA.

2. Materials and methods

Mice. Inbred B6 mice were obtained from CLEA Japan. Mito-mice-tRNA^{Lys7731} were generated in our previous work [12]. Animal experiments were performed in accordance with protocols approved by the Experimental Animal Committee of the University of Tsukuba, Japan (approval number, 14243).

Genotyping of mtDNA. To detect the G7731A mutation, a 130-bp fragment containing the 7731 site was PCR-amplified by using the nucleotide sequences from 7633 to 7653 (5′-GCCCATTGTCCT AGAAATGGT-3′) and 7762 to 7732 (5′-ACTATGGAGATTTTAAGG TCTCTAACTTTAA-3′) as oligonucleotide primers. The G7731A mutation creates a restriction site for *DraI* and generates 96- and 34-bp fragments on DraI digestion of PCR products. The restriction fragments were separated by electrophoresis in a 3% agarose gel. For quantification of G7731A mtDNA, we used ImageJ (Rasband, WS., Image J, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2014) software.

Histopathologic analyses. Formalin-fixed, paraffin-embedded sections (thickness, 5 μm) were stained with hematoxylin and eosin (H&E) to identify features characteristic of renal failures. Cryosections (thickness, 10 μm) of skeletal muscle were stained with modified Gomori trichrome for histopathologic analysis to identify RRFs. Cryosections (thickness, 10 μm) of renal tissues were prepared, and histologic analyses of COX and succinate dehydrogenase activities were performed as described previously [15].

Grip strength test. Muscle strength was estimated by using a Grip Strength Meter (Columbus Instruments, Columbus, USA); three sequential trials were performed on each mouse bilaterally.

Measurement of blood glucose, lactate, and BUN. To determine fasting blood lactate and glucose concentrations, peripheral blood was collected from the tail veins of mice after food had been withheld overnight. Glucose (1.5 g/kg body weight) was administered orally, blood was collected 15–120 min after glucose administration, and lactate and glucose concentrations were measured with an automatic blood lactate meter (Lactate Pro 2; Arkray, Kyoto, Japan) and glucose meter (Dexter ZII; Bayer, Leverkusen, Germany), respectively. BUN was measured with a Urea N B test (Wako Pure Chemical, Osaka, Japan) in accordance with the manufacturer's protocol.

Measurement of hematocrit. To determine hematocrit, capillary blood samples were obtained from each mouse by using heparinized capillary tubes, which then were centrifuged at 10,500 × *g* for 5 min. Packed cell volumes were measured by using a hematocrit reader.

Statistical analysis. Data are presented as mean ± SD and were analysed by using Student's *t* test; *P* values less than 0.05 were considered significant. Excel software was used for all statistical analysis.

3. Results

3.1. Late-onset metabolic abnormalities in aged mito-mice-tRNA^{Lys7731}

We used aged (26-month-old) male mito-mice-tRNA^{Lys7731} with low (<5%) or high (70%–75%) levels of G7731A mtDNA in their tails at 4 weeks after birth and age- and sex-matched B6 mice (negative controls). We first evaluated body length (Fig. 1A) and muscle strength (Fig. 1B), because abnormalities in these phenotypes are expressed in young (4-month-old) mito-mice-tRNA^{Lys7731} [12] and can be examined without killing the mice. Short body length (Fig. 1A) and muscle weakness (Fig. 1B), which are closely associated with the clinical abnormalities caused by the orthologous G8328A mutation in the human mitochondrial *tRNA^{Lys}* gene [13,14], occurred exclusively in mito-mice-tRNA^{Lys7731} with high levels of G7731A mtDNA. These results are consistent with our previous findings obtained from young mito-mice-tRNA^{Lys7731} [12].

We then examined various metabolic parameters relevant to mitochondrial diseases. Whereas these features were normal in our young mito-mice-tRNA^{Lys7731} [12], we expected that abnormalities in these parameters would be expressed as late-onset disorders as the mito-mice-tRNA^{Lys7731} aged. Unlike aged B6 mice and aged mito-mice-tRNA^{Lys7731} with low levels of G7731A mtDNA, aged mito-mice-tRNA^{Lys7731} with high levels of G7731A mtDNA exclusively had low hematocrit values (Fig. 1C), lactic acidosis (Fig. 1D),

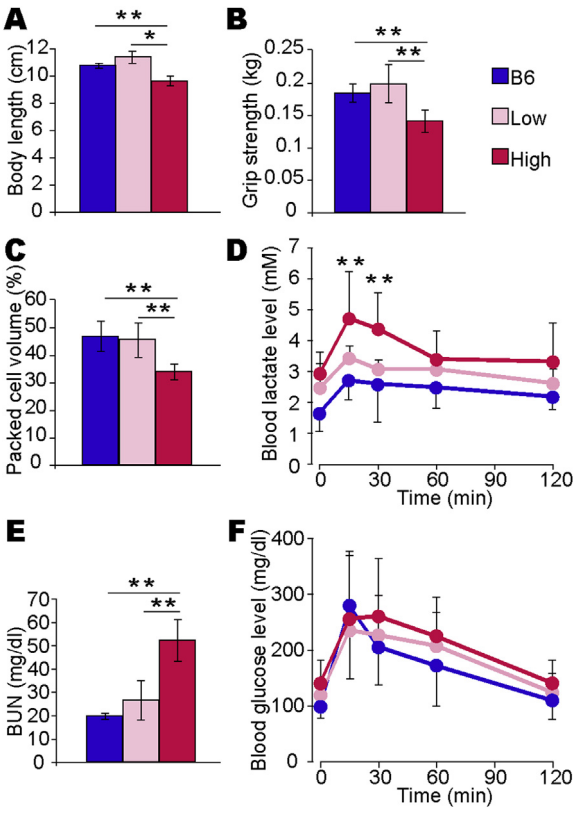

Fig. 1. Mitochondrial disease–related parameters that can be examined without euthanizing aged mito-mice-tRNA^{Lys7731}. Study populations comprised aged B6 mice ($n = 6$) and aged mito-mice-tRNA^{Lys7731} with low (less than 5% in tail tissue; $n = 4$) and high ($n = 4$; 70%, 70%, 72%, and 75% in tail tissue [Fig. S1]) levels of G7731A mtDNA in tails at 4 weeks after birth. Disease-related parameters were compared at 26 months after birth. Intergroup comparison of (A) body length, (B) grip strength, (C) hematocrit, (D) blood lactate level, (E) BUN value, and (F) blood glucose level. Data are presented as means ± 1 SD. *, $P < 0.05$; **, $P < 0.01$.