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Direct effects of mitochondrial dysfunction on poor bone health in Leigh syndrome

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ABSTRACT

Mitochondrial diseases are the result of aberrant mitochondrial function caused by mutations in either nuclear or mitochondrial DNA. Poor bone health has recently been suggested as a symptom of mitochondrial diseases; however, a direct link between decreased mitochondrial function and poor bone health in mitochondrial disease has not been demonstrated. In this study, stem cells from human exfoliated deciduous teeth (SHED) were isolated from a child with Leigh syndrome (LS), a mitochondrial disease, and the effects of decreased mitochondrial function on poor bone health were analyzed. Compared with control SHED, LS SHED displayed decreased osteoblastic differentiation and calcium mineralization. The intracellular and mitochondrial calcium levels were lower in LS SHED than in control SHED. Furthermore, the mitochondrial activity of LS SHED was decreased compared with control SHED both with and without osteoblastic differentiation. Our results indicate that decreased osteoblast differentiation potential and osteoblast function contribute to poor bone health in mitochondrial diseases.

1. Introduction

Mitochondria are organelles that produce ATP by oxidative phosphorylation. Almost all mitochondrial proteins are encoded by nuclear DNA, and are imported into the mitochondria after synthesis in the cytosol [1]. However, mitochondria are unique among organelles in that they also contain their own genome, with 13 respiratory chain subunits encoded by mitochondrial DNA (mtDNA) and synthesized in the mitochondria in humans [2]. Mitochondrial diseases such as Leigh syndrome (LS); mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); and myoclonic epilepsy with ragged red fibers (MERRF) are caused by reduced mitochondrial function caused by mutations in mitochondrial proteins encoded by either nuclear DNA or mtDNA [3–5].

The main symptoms of mitochondrial diseases manifest in the brain, skeletal muscle, and liver, which are highly dependent on their mitochondria for energy production [3–5]. In addition, manifestations of poor bone health, such as osteoporosis and osteopenia, have been suggested to be symptoms of mitochondrial diseases [5,6]. Mice with mutated mtDNA polymerase are used a mouse model of mitochondrial disease, and display symptoms of osteoporosis [7]. “Mito-mice”, another mouse model of mitochondrial disease caused by large deletions in the mtDNA genome, have decreased cortical bone thickness [8]. A recent epidemiological study also suggested that mitochondrial diseases pose a risk to bone health, with bone fractures and reduced bone density reported in 73% of patients with mitochondrial diseases [6]. It has been speculated that poor bone health associated with mitochondrial diseases is caused by secondary factors, such as endocrine dysfunction and loss of motor function due to encephalomyopathy, which is also a symptom of mitochondrial diseases [5,6]. However, whether reduced mitochondrial function has direct effects on bone health remains unknown.

LS is a progressive neurodegenerative disease that causes symmetrical necrotizing lesions in the basal ganglia and brainstem, and manifests as psychomotor retardation, muscle weakness and

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hypotonia, growth impairment, and myopathy [3,9]. The pathogenesis of LS involves reduced mitochondrial function caused by mutations in either the mtDNA or in both the nuclear DNA and mtDNA [3]. In this study, using stem cells from human exfoliated deciduous teeth (SHED), a mesenchymal stem cell [10], isolated from a patient with LS, we have analyzed the effects of reduced mitochondrial function associated with mitochondrial disease on bone formation ability, and demonstrate that optimal mitochondrial function is required for both osteoblastic differentiation and osteoblast function in these cells.

2. Materials and methods

2.1. Isolation and culture of SHED

Experiments using human samples were reviewed and approved by the Kyushu University Institutional Review Board for Human Genome/Gene Research (permission number: 678-00) and were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from the patient's guardians. Deciduous teeth were collected from a healthy control and a patient with LS at 4 and 6 years of age, respectively. SHED were isolated from dental pulp tissues as previously described [10], and grown in a culture medium consisting of Minimum Essential Medium Eagle Alpha Modification (Sigma-Aldrich, MO, USA) with 15% fetal bovine serum (Sigma-Aldrich), 100 μM L-ascorbic 2-phosphate (Wako Pure Chemical Industries, Osaka, Japan), 2 mM L-glutamine (Life Technologies, NY, USA), 100 U/mL penicillin (Life Technologies), 100 μg/mL streptomycin (Life Technologies) and 25 μg/mL Fungizone (Life Technologies) at 37 °C in 5% CO_2. SHED were used at passage 3–10. To evaluate their mesenchymal stem cell characteristics, SHED were immunostained with anti-STRO1 antibody (MAB1038; R&D systems, MN, USA), counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Dojindo, Kumamoto, Japan), and observed by fluorescent microscopy as previous described [10].

2.2. Measurement of bone mineral density

The bone mineral density of the patient with LS was measured by dual energy x-ray absorptiometry (DEXA) scanning of the lumbar spine from L2 to L4 using a Discovery A (Hologic, MA, USA) scanner.

2.3. Osteogenic differentiation of SHED

$4.5 \times 10^4/cm^2$ cells were cultured in 6-well plates to confluence. To differentiate SHED into osteoblasts, the cells were cultured in differentiation medium, which was the culture medium described above supplemented with 1.8 mM potassium dihydrogen phosphate (Nakarai tesque, Kyoto, Japan) and 10 nM dexamethasone (Sigma-Aldrich). The medium was changed twice in a week.

2.4. Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA extraction and RT-qPCR were performed as previously described [10]. The sequences of the primer sets used in this study were as follows: alkaline phosphatase (ALP), 5′-ACGTGGCTAAGAATGTCATC-3' (ALP forward) and 5′-CTGGTAGGCGATGTCCTTA-3' (ALP reverse); and 18S rRNA, 5′-CGGCTACCACATCCAAGGAA-3' (18S rRNA forward) and 5′-GCTGGAATTACCGCGGCT-3' (18S rRNA reverse). The relative expression levels of the target genes were analyzed using the comparative threshold cycle method by normalizing to 18S rRNA.

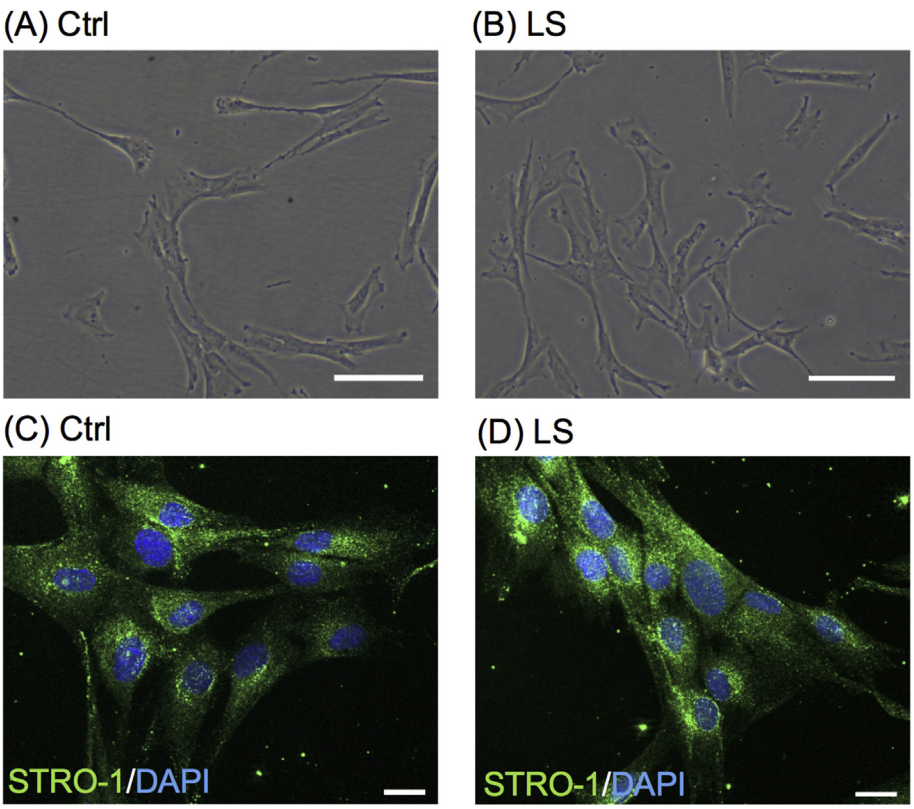

Fig. 1. Isolation and characterization of SHED from a patient with LS.
(A, B) Isolated SHED were observed with a phase-contrast microscope. Scale bar = 100 μm. (C, D) SHED were stained with an anti-STRO1 antibody and DAPI. Scale bar = 20 μm.

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