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Treadmill running induces satellite cell activation in diabetic mice

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ABSTRACT

Skeletal muscle-derived stem cells, termed as satellite cells, play essential roles in regeneration after muscle injury in adult skeletal muscle. Diabetes mellitus (DM), one of the most common metabolic diseases, causes impairments of satellite cell function. However, the studies of the countermeasures for the DM-induced dysfunction of satellite cells have been poor. Here, we investigated the effects of chronic running exercise on satellite cell activation in diabetic mice focused on the molecular mechanism including Notch and Wnt signaling, which are contribute to the fate determination of satellite cells. Male C57BL/6 mice 4 weeks of age were injected with streptozotocin and were randomly divided into runner group and control group. Runner group mice were performed treadmill running for 4 weeks. DM attenuated satellite cell activation and the expressions of the components of Notch and Wnt signaling. However, chronic running resulted in activation of satellite cells in diabetic mice and salvaged the inactivity of Wnt signaling but not Notch signaling. Our results suggest that chronic running induces satellite cell activation via upregulation of Wnt signaling in diabetic as well as normal mice.

1. Introduction

Muscle-specific stem cells, termed as satellite cells, are located between the basal lamina and plasma membrane of muscle fibers [1]. Satellite cells are mitotically quiescent under normal physiological conditions but are activated in response to stimulation, such as muscle injury, and proliferate extensively. Majority of satellite cells differentiate into mature muscle fibers, whereas others return to a quiescent state to self-renew and maintain the stem cell pool. In adult skeletal muscle, satellite cells play an essential role in muscle regeneration and maintain the plasticity of the skeletal muscle [2,3].

Diabetes mellitus (DM) is one of the most common metabolic diseases worldwide, and the number of patients with DM increased in recent years. Patients with DM exhibit hyperglycemia caused by impairments in insulin secretion (type 1), or action (type 2), or both. Type 1 DM is characterized by an immune-mediated destruction of β cells in the pancreatic islets of Langerhans, leading to insulin deficiency [4]. It is well known that type 1 DM is developed in childhood and can lead to severe long-term complications including retinopathy, neuropathy and nephropathy [5]. On the other hand, type 2 DM occurs through mechanisms such as insulin resistance in peripheral tissues and increased blood glucose levels caused by overnutrition [6,7]. DM is often associated with the development of secondary complications in various organs, such as eyes, kidneys, heart, brain, and skeletal muscle [8]. Previous studies have reported that DM induces a variety of alterations in the structure and function of the skeletal muscle, such as muscle atrophy [9], fiber-type transition [10], muscle weakness [11], and a decline in energy metabolism [12]. In addition, DM attenuates satellite cell function, including proliferation, differentiation, and subsequent muscle regeneration. Satellite cells derived from diabetic mice have an impaired ability to differentiate into myotubes [13]. The delay of regeneration after muscle injury was observed in several models of DM such as *Akita*, *ob/ob*, and *db/db* mice [14,15]. Although the effects of DM on the satellite cell function have been extensively investigated, not many studies have focused on countermeasures for diabetes-induced attenuation of satellite cells, and the molecular mechanisms underlying the changes remain unclear.

One of the candidates for the countermeasure is physical exercise, which contributes to satellite cell activation and proliferation. The increments in the number and proliferative ability of satellite cells have been previously reported in both human and animal studies [16,17]. Physical exercise also induces the change in

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extracellular signaling in the skeletal muscle that affects satellite cells. For instance, Notch signaling, which is involved in cell fate choice and regulates satellite cell proliferation, is activated by exercise according to increased expressions of ligands, Notch receptors, and downstream effectors in myogenic cells [18,19]. Conversely, exercise-induced upregulation of Wnt signaling, which contributes to satellite cell activation and lineage specification in the skeletal muscle, has been reported [20,21]. However, it remains unclear whether physical exercise can prevent satellite cell dysfunction, including the activities of Notch and Wnt signaling, by DM.

In the present study, we investigated the molecular mechanisms of satellite cell activation by chronic running exercise in diabetic mice with a focus on the Notch and Wnt signaling pathway. We used type 1 DM model mice generated by intraperitoneal administration of streptozotocin, which is a compound that displays a preferential toxicity toward pancreatic β cells. We found that DM decreased the number of satellite cells and inhibited satellite cell activation via downregulation of Notch and Wnt signalings. However, chronic running improved Wnt signaling activity, but not Notch signaling in diabetic mice. Of note, chronic running increased the number of activated satellite cell in diabetic as well as normal mice. Thus, these results may provide future perspectives on exercise-based medicine as a countermeasure for DM-induced dysfunction of satellite cells.

2. Materials and methods

2.1. Animals

Experiments were performed on 4-week-old male C57BL/6 mice (Japan SLC Inc., Hamamatsu, Japan) weighing 19–21 g. DM was induced by a single intraperitoneal injection of 200-mg/kg streptozotocin (STZ, Wako) dissolved in citrate buffer. The blood glucose levels were measured 1 week after injection, and mice with blood glucose levels higher than 300-mg/dL were considered diabetic as previously described [22]. Mice were randomly divided into four groups (n=5 per group): control (Cont), runner (Run), diabetes (DB), and diabetes/runner (DB+Run). Mice in the DB and DB+Run groups were injected with STZ, and mice in the Cont and Run groups were injected with an analogous volume of citrate buffer. Animals were housed in standard cages in facilities with controlled temperature and humidity under a 12:12-h light/dark cycle and had free access to chow and water. Animal experiments were performed in a humane manner after receiving approval from the Institutional Animal Care and Use Committee of the National Institute of Advanced Industrial Science and Technology.

2.2. Treadmill running and tissue sampling

Animals in the Run and DB+Run groups performed treadmill running (10–20 m/min, 60 min/day, 5 days/week) for 4 weeks starting 1 week after injection of citrate buffer or STZ. After 4 weeks of running, the mice were sacrificed by cervical dislocation. The plantaris (for biochemical analysis) and gastrocnemius (for immunohistochemistry) were dissected from each mouse and frozen in liquid nitrogen after measuring the wet weight and stored at −80 °C until analysis.

2.3. Immunohistochemistry (IHC)

Cross-sections of the midportion of the gastrocnemius were cut at 10 μm in a cryostat (Microm Cryo-Star HM 560, Walldorf, Germany) and maintained at −20 °C until analyses. The sections were fixed in 0.1 M phosphate buffer containing 4% paraformaldehyde for 5 min and degreased in 100% methanol for 10 min at −20 °C. After being washed by phosphate-buffer saline (PBS), the sections were blocked in 10% donkey serum diluted with PBS containing 0.1% Triton-X 100 (PBS-T) for 20 min. Then, the sections were incubated overnight at 4 °C with anti-Laminin α2 (1:600; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Pax7 (1:100; Developmental Studies Hybridoma Bank, Iowa City, IA, USA) and anti-MyoD (1:200; Santa Cruz Biotechnology) antibodies diluted in PBS-T containing 1% bovine serum albumin (BSA). Immunoreactivity was detected by incubation with Cy3-conjugated donkey anti-mouse IgG (1:500; Jackson ImmunoResearch, West Grove, PA, USA) and AlexaFluor 488-conjugated donkey anti-rabbit IgG (1:500; Life Technologies, Carlsbad, CA, USA) diluted in PBS-T containing 1% BSA for 4 h, Sections were counterstained with 4′,6-diamidino-2-phenylindole (Wako Pure Chemical Industries, Osaka, Japan). After several washes, the stained sections were mounted using the mounting medium (KPL, Gaithersburg, MD, USA). Images were acquired using an Olympus FV1000-D confocal microscope (Olympus, Tokyo, Japan).

2.4. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was isolated from frozen plantaris muscles using Isogen. RNA samples were treated with Turbo DNase to remove genomic DNA. cDNA synthesis was performed using PrimeScript RT Master Mix (Takara Bio., Otsu, Japan) according to the manufacturer's recommendations. qRT-PCR analysis were performed as previously described [23] and specific primers obtained from Life Technologies (Table 1).

2.5. Protein extraction and Western blot analysis

Protein extraction from plantaris muscles and Western blot analysis were performed as previously described [23].

2.6. Statistical analysis

Data were analyzed using the Student's *t* test and were expressed as mean ± SE. P values < 0.05 were considered to be statistically significant.

3. Results

3.1. STZ-induced diabetes gives rise to skeletal muscle atrophy

Intraperitoneal injection of STZ resulted in the development of pronounced hyperglycemia in experimental animals [24]; the blood glucose levels of all mice in the DB and DB+Run groups were greater than 300 mg/dL 1 week after injection. In a previous study, STZ-induced diabetes resulted in severe muscle atrophy [25]. Accordingly, body weight and wet weights of the plantaris and gastrocnemius muscles of the DB group were significantly lower than that of the Cont group at the end of the experiment (Fig. 1A–C). Four weeks of treadmill running did not affect on the muscle weights in both normal and diabetic mice, as indicated by the unchanged wet weights of the plantaris and gastrocnemius muscles to body mass ratio in the trained animals compared with those of the sedentary animals (Fig. 1A–C). The cross-sectional areas of gastrocnemius muscles were also lower in diabetic mice (Fig. 1D, E) regardless of performing exercise. These data suggest that STZ-induced diabetes causes skeletal muscle atrophy and chronic running cannot salvage the phenotype.