International Journal of Biological Macromolecules 117 (2018) 287–293

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Effects of functional β-glucan on proliferation, differentiation, metabolism and its anti-fibrosis properties in muscle cells

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ARTICLE INFO

Article history:
Received 22 February 2018
Received in revised form 21 May 2018
Accepted 25 May 2018
Available online 26 May 2018

Keywords:
β-glucan
Muscle
C2C12

ABSTRACT

Skeletal muscles plays a crucial role in metabolism and exercise. Fuctional β-glucan is polysaccharide that is found in the cell walls of cereal, which is known to reduce cholesterol and lipid, prevent diabetes, cancer and cardiovascular diseases. In an attempt to identify β-glucan that could promote skeletal muscle function, we analyzed the proliferation, differentiation, metabolism and anti-fibrotic properties of β-glucan in C2C12 muscle cells. Treatment of β-glucan in C2C12 myoblasts led to increased proliferation and differentiation. Besides that, we found that C2C12 myotubes treated with β-glucan displayed a fast-to-slow muscle fiber conversion and improved oxidative metabolism. Further study revealed that β-glucan treatment could prevent myotubes from becoming myofibroblasts. Together, our study suggests that functional β-glucan might have a therapeutic potential to improve skeletal muscle function, which might contribute to the development of β-glucan.

1. Introduction

Skeletal muscle, also known as striated muscle, most attached to the bones, is an important exercise organs. The body's movement and maintain posture are closely related to the normal function of skeletal muscle [1]. At the same time, skeletal muscle also plays a pivotal role in metabolism. Since skeletal muscle has a relatively large mass and energy requirement, which is a major site for glucose utilization, fatty acids oxidation and protein storage [2]. Therefore, the abnormal of skeletal muscle exercise and metabolism is usually closely related to many metabolic disease [3].

It is widely accepted that mammalian skeletal muscles are composed of four different types of fiber with different contractile and metabolic properties [4]. Myofiber types are determined by different myosin heavy chain (MyHC) subtypes, which could be simply divided into two groups: i) fast-twitch muscle (MyHC IIa, MyHC IIb and MyHC IIx), also known as white muscle, dominated by glycolysis, play a role in short-term exercise; ii) slow-twitch muscle (MyHC I), also known as red muscle, riched in oxidase, play a role in long-term exercise [4–6]. Of note, slow-twitch muscle possess higher oxidative capacity to maintain metabolic homeostasis [7]. It is known that myofiber types in obesity or diabetes will shift from slow to fast [8].

Meanwhile, skeletal muscle cell has considerable capacity to proliferate, differentiate and regenerate following injury or damage [9,10]. The activation and proliferation of myogenic satellite cell is considered the first step in skeletal muscle regeneration. Then the myoblasts enter the stage of differentiation to form myotubes. However, the exercise and biomedical properties of recovered skeletal muscle are impaired due to the presence of fibrotic tissues, which largely depends on the TGFβ/Smad fibrosis signaling pathway [11]. Therefore, treatment promoting muscle cells regeneration and against skeletal muscle fibrosis is required.

Functional β-glucan, also known as (1, 3)(1, 4)-β-D-glucan, is polysaccharide that is found in the cell walls of cereal, such as oat, barley, yeasts and bacteria [12]. It is well established that β-glucan reduces serum cholesterol and lipid levels, prevent diabetes, cancer and cardiovascular diseases [13–15]. As selective substrates for gut microbiota,

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https://doi.org/10.1016/j.ijbiomac.2018.05.188
0141-8130/

Table 1
Primer sequences for RT-PCR.

Gene	Sense sequence (5′ → 3′)	Antisense sequence (5′ → 3′)
Smad4	CGGCCGTGGCAGGGAACA	CTGCAGAGCTCGGTGAAGGTGAAT
Col I	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
VIM	CGGCTGCGAGAGAAATTGC	CCACTTTCCGTTCAAGGTCAAG
α-SMA	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA
TGF-β	TGACGTCACTGGAGTTGTACGG	GGTTCATGTCATGGATGGTGC
MyHC I	CTGCCTCTGCACCCCATAATG	TTGCTGAGATGACAGAACGCT
MyHC IIa	CAGCTGCACCTTCTCGTTTG	CCCGAAAACGGCCATCT
MyHC IIb	CAATCAGGAACCTTCGGAACAC	GTCCTGGCCTCTGAGAGCAT
MyHC IIx	GGACCCACGGTCGAAGTTG	CCCGAAAACGGCCATCT
P21	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
CCND1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
MyoG	AGTGAATGCAACTCCCACAG	ACGATGGACGTAAGGGAGTG
MyoD	CGCCACTCCGGGACATAG	GAAGTCGTCTGCTGTCTCAAAGG
MEF2C	GTCAGTTGGGAGCTTGCACTA	CGGTCTCTAGGAGGAGAAACA
Mox2	TGTCCTACCCCGAACTCTCC	GTGCCAGTTGCTTTGCAGA
Myf5	AAGGCTCCTGTATCCCCTCAC	TGACCTTCTTCAGGCGTCTAC
18 s	ACCGCAGCTAGGAATAATGGA	CAAATGCTTTCGCTCTGGTC

consumption of β-glucan favorably alters the composition of microbiota and reduced cardiovascular diseases risk [16,17]. However, the function and effect of β-glucan on skeletal muscle or myoblast is still not clear.

Here, the effects of β-glucan on the muscle cell metabolism, proliferation, differentiation and fibrosis were investigated. Based on our *in vitro* results, we found that C2C12 myotubes treated with β-glucan displayed a fast-to-slow muscle fiber conversion and improved oxidative metabolism. Further study revealed that, β-glucan could promote proliferation and differentiation of C2C12 myoblasts, while β-glucan treatment could prevent myotubes from becoming myofibroblasts. The identification of the new function of β-glucan in skeletal muscle cells may provide more information for the development of functional β-glucan and novel strategies for improving muscle function.

2. Methods & materials

2.1. Cell culture

Mouse C2C12 myoblast cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Gibco), and 100 units/mL penicillin, and 100 mg/mL streptomycin. For induction of myoblasts into myotubes, when C2C12 myoblasts reached 80–90% confluence, cells were differentiated into myotubes in differentiation medium, consisting of DMEM containing 2% HS, and 1% P/S. To determine the effects of functional β-glucan in C2C12 myotubes which have been differentiated for 4 d. For functional β-glucan treatment, β-glucan from barely (G6513, Sigma Aldrich) was dissolved in the culture medium (20 mg/mL), then the myoblasts or myotubes were incubated with β-glucan as indicated concentration for 24 h before harvest. The structure, molecular weight and viscosity of functional β-glucan were shown in Fig. S1. For TGFβ treatment, 20 ng/ml TGFβ (Santa cruz) stimulated the cells individually and costimulated cells with 20 mg/mL β-glucan for 12 h.

2.2. Western blot & qRT-PCR

Cultured cells were lysed in RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1.0 mM EDTA, 0.1% SDS, 1% Sodium deoxycholate, and 1% Triton X-100), which containing Protease Inhibitor Cocktail and Phosphatase Inhibitor Cocktail (Roche). The protein concentration was determined using a BCA/Bradford protein assay kit (Thermo Fisher). Protein samples were resolved on 10% SDS-PAGE gels by using standard procedures. Anti-p21 (Santa Cruz), anti-cyclin D1 (Santa Cruz), anti-MyoG (Santa Cruz), anti-p-Akt-S473 (Cell signaling), anti-FoxO1 (Cell Signaling), anti-FoxO3 (Cell Signaling), anti-P-FoxO1 (Cell Signaling), anti-PDK4 (Proteintech), anti-Smad4 (Cell signaling), anti-Col I (Abcam), anti-Vimentin (Abcam), anti-α-SMA (Cell signaling) anti-Tubulin (Sigma Aldrich) and anti-GAPDH (Sigma Aldrich) were used for western blot analysis.

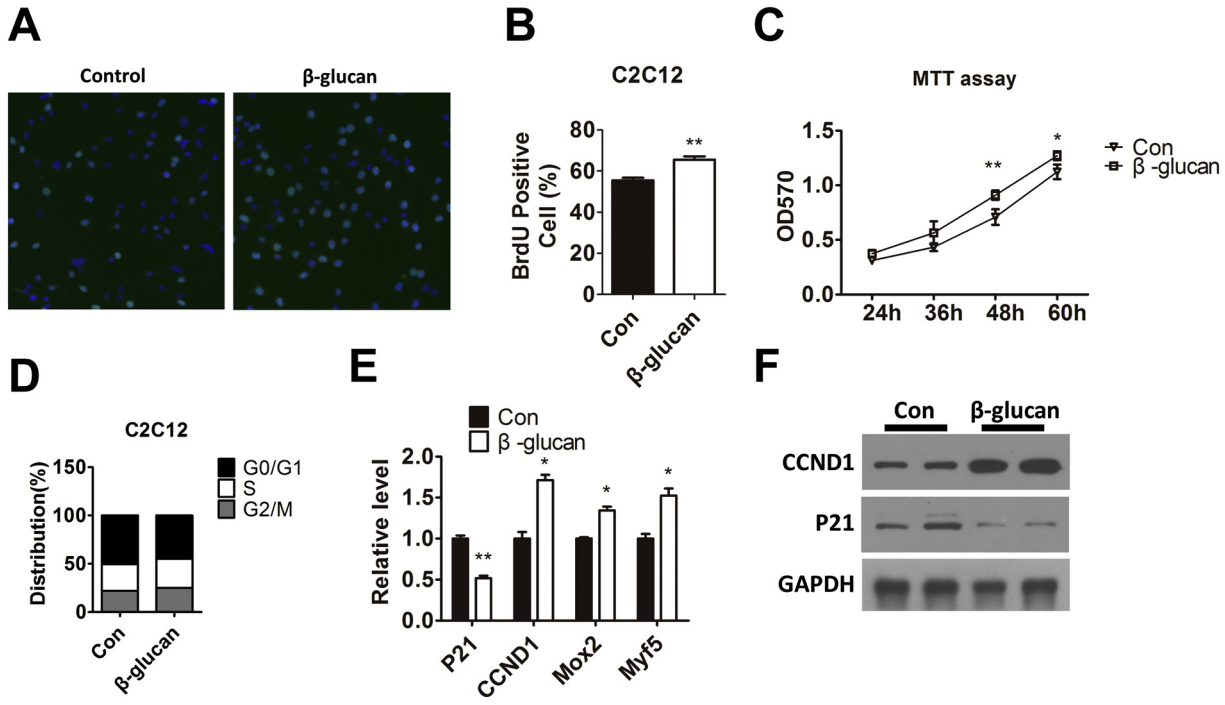

Fig. 1. Functional β-glucan promotes C2C12 myoblasts proliferation. (A & B) Proliferation of C2C12 myoblasts was evaluated by BrdU staining. Cells were treated with functional barely β-glucan for 24 h. Representative images of cells were taken by fluorescence microscope (A). The percentage of BrdU positive cells was measured (B). (C) Growth curves of C2C12 myoblasts treated with β-glucan were measured by MTT assay. (D) Cell cycle analysis of C2C12 myoblasts treated with β-glucan as indicated. (E) RT-PCR analysis of Myf5, Mox2, cyclin D1 and p21 mRNA levels in C2C12 myoblasts treated with β-glucan for 24 h. (F) Western blot analysis of cyclin D1 and p21 protein levels in C2C12 myoblasts treated with β-glucan for 24 h. GAPDH was used as a loading control. Data are mean ± SEM, which are from three typical independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.