SUPPLEMENTARY MATERIAL

Agaricus bisporus-derived β-glucan enter macrophages and adipocytes by CD36 receptor

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Abstract

β-glucans are a heterogeneous group of natural polysaccharides. They are ubiquitously found in bacterial or fungal cell walls, cereals, seaweed, and mushrooms. The beneficial role of β-glucan in tumor, insulin resistance, dyslipidemia, hypertension, and obesity is being continuously documented. Ample evidence showed that β-glucan could act on several receptors, such as Dectin, complement receptor (CR3), TLR-2, 4, 6 and scavenger. Based on the above, we wanted to explore whether agaricus bisporus-derived β-glucan acted on these receptors on Raw 264.7 macrophages and 3T3-L1 adipocytes.

Keywords: agaricus bisporus-derived β-glucan, Raw 264.7 macrophages, 3T3-L1 adipocytes, CD36, imaging flow cytometry
Experimental

Drugs and antibodies

Dexamethasone (Dex), 3-isobutyl-1-methylxanthine (IBMX), insulin (Ins) were purchased from Sigma (St. Louis, MO, USA). The primary antibodies used in this experiment were CD36 (1:100), F4/80 with Alexa Fluor 624 (1:100), and secondary antibody Alexa Fluor 405 (1:200) were purchased from Abcam.

Cells culture

Raw 264.7 cells and 3T3-L1 fibroblasts were maintained in DMEM supplemented with 10% FBS, 50 units /ml penicillin, and 50 μg /ml streptomycin in a humidified atmosphere (5% CO₂) at 37°C.

3T3-L1 cells were differentiated into adipocytes as followings: 2 days after reaching confluence, the cells were cultured in DMEM containing Dex 1μM, IBMX 0.5mM, Ins 5μg/ml for 2 days, followed by culture in maintenance medium (DMEM with 10% FBS, and Ins 5μg/ml) for 2 days, and thereafter, Ins was removed from the medium for 4 days (Kurokawa, Arai et al. 2010). 3T3-L1 cells are differentiated to adipocytes on 8 days.

Treatments for flow cytometry

Raw 264.7 cells and induced 3T3-L1 adipocytes were incubated with CD36 ab for 4h, and then washed with medium for 3 times, FITC-β-glucan (5μg/ml) was added to the medium for 1h. The cells were blocked with 1% BSA+10% goat serum for 1h. After that, the cells were incubated with Alexa Fluor 405 for 4h. As for control, cells were incubated with FITC-β-glucan (5μg/ml) for 1h, and then washed with medium for 3 times, F4/80-Alexa Fluor 647 or Nile Red were added to the medium for 4h, after that, cells were exploded to Dapi (solarbio, China) for 10min. Finally, these cells were suspended with PBS in EP avoid light. The distributions of FITC-β-glucan in these two cells were detected by flow cytometry (Flowsight, Merk Millipore, USA).

Flowsight data acquisition and analysis

Instrument was adjusted according to procedure (Terrazas, Oghumu et al. 2015). Acquisition speed was set up to low speed and the highest resolution. Cells were acquired based on area and aspect ratio, gating out single cells from the analysis. About 5000 cells were acquired. Channel 2 was used to acquire FITC-β-glucan, channel 4 was used to detect
Nile red, channel 7 was used to detect DAPI or CD36-Alexa 405 and channel 11 was used to detect F4/80-Alexa 647. Data were analyzed in IDEAS software after compensation of single color control samples using a compensation matrix. FITC-β-glucan internalized by Raw 264.7 macrophages and 3T3-L1 adipocytes, and colocalization of FITC-β-glucan with Nile red or F4/80 was analyzed using the internalization and colocalization analysis application Wizard in IDEAS software. To evaluate the CD36 as the receptor of β-glucan, the intensity of FITC-β-glucan with or without CD36 neutralizing antibody was compared in IDEAS software.

**Treatment for HPLC**

The content of β-glucan was detected by high-performance lipid chromatography (HPLC) according to Chinese Pharmacopoeia about yeast β-glucan. Briefly, HPLC separation was performed on waters sugar pak-1 columns (6.5 mm×300 mm) and detected using refractive index detector. The mobile phase was water. The column temperature was kept at 80 °C and the flow rate was set at 0.5 mL/min. The total protein of cellular contents was quantified by BCA protein assay kit (Tiangen, Beijing).
**Figure S1** The take-up and distribution of β-glucan of Raw 264.7 macrophages.

The representative images of Raw 264.7 and the ratio of FITC-β-glucan-internalized (A) and FITC-β-glucan-nuclear-localized (B) cells to the double staining cells, and the co-localization (C) of FITC-β-glucan and F4/80 cells to the double staining cells.
Figure S2 The take-up and distribution of β-glucan on 3T3-L1 adipocytes.

The representative images of 3T3-L1 adipocytes and the ratio of FITC-β-glucan -internalized cells to the double staining cells (A), FITC-β-glucan –nuclear localized cells to the double staining cells (B), and FITC-β-glucan co-located with Nile red cells to the double staining cells (C).
Figure S3 The mRNA expression of β-glucan related receptors in Raw 264.7 macrophages and 3T3-L1 adipocytes.

Figure S4 The HPLC of Raw 264.7 macrophages cellular contents treated by DMEM +
FITC-β-glucan, and DMEM + CD36 antibody + FITC-β-glucan.

The protein concentration of RAW 264.7 macrophages treated by FITC-β-glucan and CD36 ab + FITC-β-glucan was 7.65mg/ml, 7.31mg/ml respectively.

Figure S5 The HPLC of 3T3-L1 adipocytes cellular contents treated by DMEM + FITC-β-glucan, and DMEM + CD36 ab + FITC-β-glucan.

The protein concentration of 3T3-L1 adipocytes treated by FITC-β-glucan and CD36 ab + FITC-β-glucan was 5.97mg/ml, 6.9mg/ml respectively.

(Terrazas, Oghumu et al. 2015)
Reference

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