EFFECT OF YEAST-DERIVED PEPTIDES ON SKELETAL MUSCLE FIBER TYPES IN C2C12 MYOTUBE CELLS

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I. INTRODUCTION

Skeletal muscle fibers are composed of slow-twitch and fast-twitch fibers according to their contraction capacity and energy metabolism characteristics. The type of muscle fibers plays an essential role in regulating muscle function and exercise endurance [1]. In our previous study, the novel antioxidant peptides (XHY69AP, AP-D, YPLP, and AGPL) were obtained from potential probiotic yeast (*Yamadazyma triangularis*) which was selected by our lab from dry-cured ham [2]. This work was designed to explore the effect of yeast-derived peptides on skeletal muscle fiber types in C2C12 cells.

II. MATERIALS AND METHODS

Cell Culture, Differentiation, and Peptide Treatment: The C2C12 cells were incubated in Dulbecco's Modified Eagle Medium (DEME) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin-streptomycin. When confluent cultures reached 80%, DMEM supplemented was changed to 2% horse serum for 4 d to initiate differentiation. Then, 100 μg/ml of yeast-derived peptides (XHY69AP, AP-D, AGPL, and YPLP) were added to the cell culture medium and incubated for 24 h. The peptide was replaced by DMEM as the control group and by L-carnosine as the positive group.

Immunofluorescence: C2C12 myotubes were immobilized in 4% paraformaldehyde, permeabilized in 0.5% Triton X-100, and treated with primer antibodies (slow MyHC and fast MyHC). Then, the myotubes were incubated with Alexa Fluor 488 secondary antibody and the nucleus was dyed by 4',6-diamidino-2-phenylindole. All images were captured by laser scanning confocal microscopy.

Real-time Quantitative PCR: Real-time quantitative PCR was carried out on a QuantStudio 6 flex PCR system with fluorescent dye. The glyceraldehyde-3-phosphate dehydrogenase was regarded as an internal reference. The expression of the target gene was analyzed according to the $2^{-\Delta\Delta CT}$ method.

Western Blotting: Western blotting analysis was performed followed by the method described by Hou *et al.* [3]. The aimed proteins were incubated with primary antibodies (slow MyHC and fast MyHC, Sigma, 1:5000) and horseradish peroxidase conjugated secondary antibody.

Statistical Analysis: The results were shown as the mean ± standard error. All data were analyzed by SPSS 20.0 software (Chicago, IL, USA), and significant differences ($p < 0.05$) were determined by Duncan's multiple-range test in one-way analysis of variance and Student's *t*-test.

III. RESULTS AND DISCUSSION

The treatment of yeast-derived peptides increased the proportion of slow-twitch fibers and decreased the proportion of fast-twitch fibers compared to the control group (Fig.1). Meanwhile, peptide supplements significantly up-regulated slow MyHC expression and down-regulated fast MyHC expression than the control group ($P<0.05$, Fig.2A). A similar result was shown for the mRNA expression of muscle fibers (Fig.2B). XHY69AP, AP-D, and YPLP improved the expressions of the slow-twitch gene (MyHC I), while they markedly down-regulated fast-twitch relative genes (MyHC Ⅱa, Ⅱb, and Ⅱx). YPLP showed a stronger increase in MyHC I expression level, which was 1.3-fold higher than control. Importantly, slow-twitch fiber contracts slowly and has a strong exercise tolerance, while

fast-twitch fiber contracts quickly and fatigues easily [1]. Thus, yeast-derived peptides promoted the transformation of fast-twitch to slow-twitch fiber, indicating the potential to improve fatigue resistance.

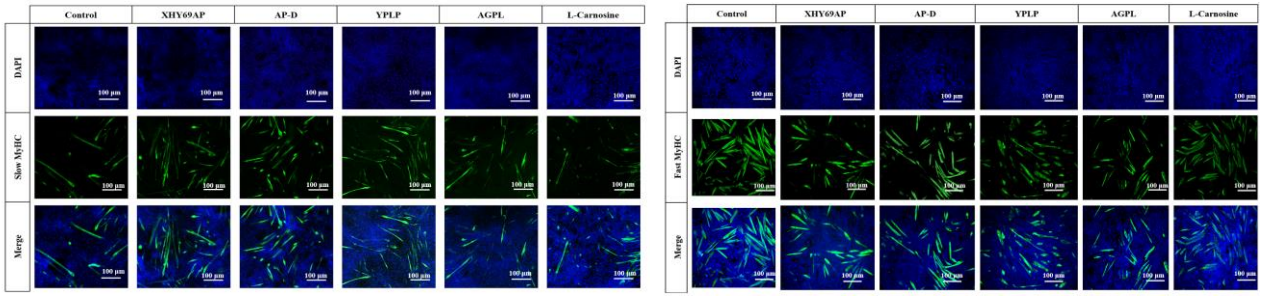

Figure 1. Immunofluorescence of slow-twitch and fast-twitch fibers.

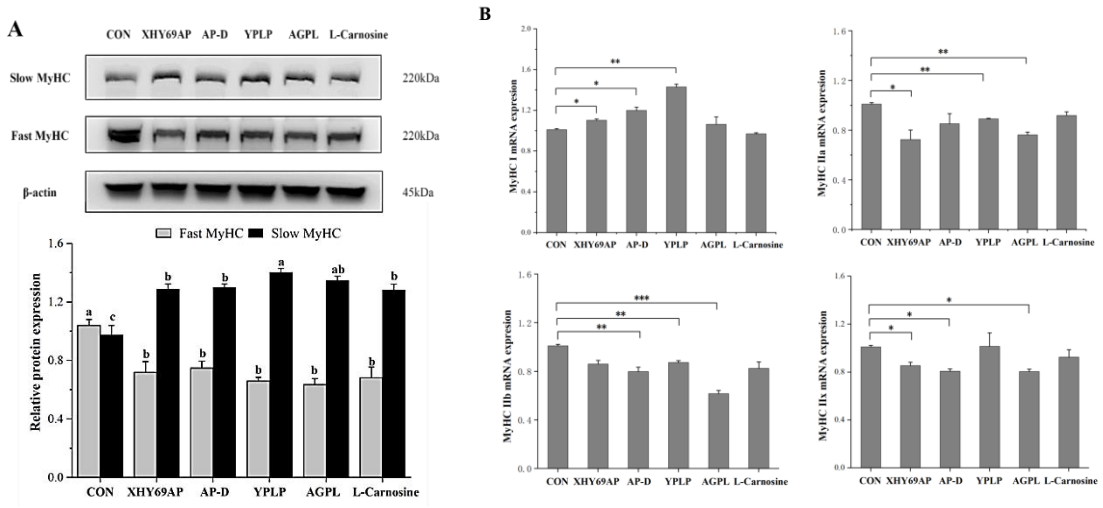

Figure 2. The expression of slow-twitch and fast-twitch fibers in protein level (A) and mRNA level (B). Different letters in the same index represent significant differences between different groups ($p < 0.05$). *$p<.05$, **$p<.01$, and ***$p<.001$ as compared with control group.

IV. CONCLUSION

In summary, yeast-derived peptides promoted the generation of slow-twitch fibers and decreased the proportion of fast-twitch fibers, potentially contributing to enhancing exercise tolerance. Further research is being conducted to further reveal the effect and mechanism of yeast-derived peptides on relieving muscle fatigue *in vivo*.

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