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- Summer Research Internship
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Relationship between AMPK-dependent BDNF pathway and KLF15 on fatty acid oxidation in skeletal muscle

Introduction

Skeletal muscle is known for its metabolic flexibility in switching myocellular fuels according to various nutritional circumstances [1]. Brain-derived neurotrophic factor (BDNF) is a metabolism-regulating myokine that is upregulated in skeletal muscle after fasting (Fast) to enhance FAO through the TrkB/ERK/AMPK pathway and downregulated after high-fat diet (HFD) due to raised mitochondrial burden [1]. Muscle Kruppel-like factor 15 (KLF15) has been to be metabolically significant in the systemic lipid homeostasis [2]. Studies have shown that Klf15 expression level in skeletal muscle increases robustly after Fast [3, 4, 5]. Although the change in Klf15 expression after HFD has not been well studied, it is found that a mutation at the zinc-finger binding motif of KLF15 in mice demonstrates resistance to fat gain [6]. It is hypothesized that BDNF pathway induces Klf15 expression to control metabolic gene expression in skeletal muscle.

Stage I

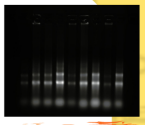
- To explore the possible functional correlation between the BDNF and KLF15-mediated lipid metabolism in mammalian skeletal muscle, thigh tissue samples from muscle-specific BDNF knockout mice (MBKO) and BDNF-floxed mice (F_1/F_1) were collected to measure the Klf15 expression level.
- To investigate the effect of caloric restriction and high-fat nutrition, six treatment groups (Fed, Fast, and HFD) of female mice (n=4) were studied. Real-time PCR results of the six groups were compared to reveal the relative fold-change in the Klf15 expression level.

Stage II

- Designed to mimic the muscle tissue environment and to study whether BDNF-KLF15 correlation occurs in an autocrine manner in vitro.
- C_2C_{12} myoblasts is a glycolytic muscle cell line that was isolated from a female mouse [1].
- To observe the myotube differentiation efficiency, adenovirus mediated BDNF silencing efficiency, intensity of green fluorescence protein, and the Klf15 expression level.

Methodology

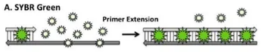
Total RNA Extraction



MBKO and F_1/F_1 female mice were respectively fed with a chow diet, HFD (3 months) and Fast (24 hrs). RNA concentration and integrity was examined by agarose gel electrophoresis and nano-spectrophotometer.

RT-PCR & real-time PCR

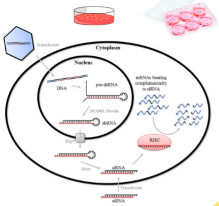
Forward: ACAGGCGAGAAGCCCTTT
Reverse: CATCTGAGCGGGAAAACCT



Designed a pair of Klf15 primers according to reference [2]. RT-PCR and real-time PCR were performed to measure the Klf15 and Rpl7 expression levels, where Rpl7 was used as the control due to its housekeeping function.

C_2C_{12} cell culture

- C_2C_{12} myotubes treated with DMEM feeding medium & horse serum differentiation medium.
- Ad-shBDNF added at day-5 of differentiation (Ad-Scram as control).
- Hank's balanced salt solution (HBSS) and palmitic acid (PA) added 6 hours and 24 hours respectively to mimic Fast and HFD conditions.



Results

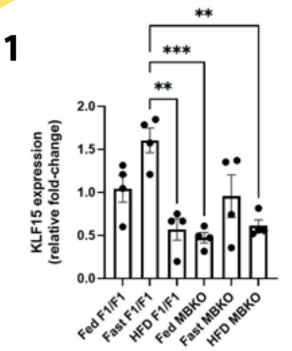


Figure 1. Klf15 expression in MBKO and F_1/F_1 mice fed with normal chow, fasting, and high-fat diet conditions. (n=4)

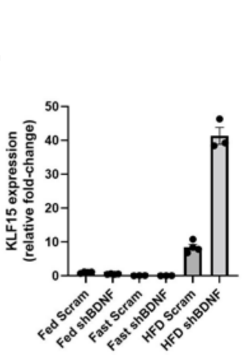


Figure 2. Klf15 expression in shBDNF and original C_2C_{12} myotubes under normal medium, fasting and high-fat diet conditions. (n=4)

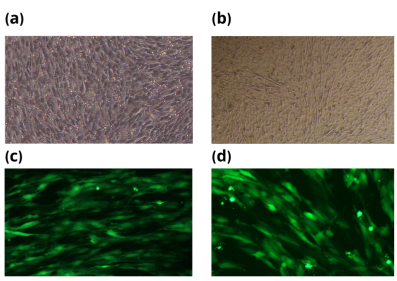


Figure 3(a). Microscopic view of one well of C_2C_{12} myoblasts at the 2nd day after seeded into a 6-well plate.

Figure 3(b). 4X microscopic view of one well of C_2C_{12} myotubes at D-8 of differentiation.

Figure 3(c). GFP intensity and myotube morphology of C_2C_{12} added with Ad-control of BDNF.

Figure 3(d). GFP intensity and myotube morphology of C_2C_{12} added with Ad-shBDNF of BDNF.

Discussion

I. Effect of MBKO, caloric restriction, and high-fat diet on Klf15 expression

BDNF-knockout
- Klf15 expression was generally downregulated when in Fed and Fast MBKO, but not in HFD (Fig. 1).
- The difference in Klf15 expression in F_1/F_1 and MBKO mice suggests that there is a correlation between the BDNF and KLF15 pathways in skeletal muscle.

Fasting
- Among F_1/F_1 and MBKO mice, fasting upregulated the Klf15 expression for a +0.5 relative fold-change, coherent to [3, 4, 5].
- Compensated expression in Fast MBKO suggests that there was a combined effect of BDNF-knockout and Fast.

High-fat diet
- Downregulated Klf15 expression, yet the same pattern was not observed among the MBKO pair. Resemblance between KLF15 and BDNF in response to HFD [1].

II. Different patterns observed in C_2C_{12} myotubes

BDNF-silencing ('shBDNF')
- Downregulated Klf15 expression after Fed (Fig. 2).
- Inconsistency in Fast and HFD groups.

Fasting
- Downregulated Klf15 expression (unpaired t-test).
- KLF15 acts collaboratively in liver and skeletal muscle [5]: extremely low Klf15 expression in muscle solo play in vitro

High-fat diet
- On the contrary, 10-fold upregulated in HFD Scram and 40-fold upregulated in HFD shBDNF.
- HFD and shBDNF have a synergistic effect.
- KLF's FAO machinery.
- BDNF role in suppressing KLF15 in preventing mitochondrial burnout.

Conclusion

This experiment involves collecting thigh tissues from BDNF F_1/F_1 and MBKO mice, which were treated with chow diet, fasting, and high-fat diet, and measuring the Klf15 expression level using RT-PCR and real-time PCR. Klf15 was downregulated by BDNF-knockout and HFD effect but upregulated by Fast. While the results showed inconsistency in C_2C_{12} myotubes in vitro set-up, the results can only draw a conclusion on the correlation between the AMPK-ACCB-PPARγ- pathway of BDNF and KLF15-PPARa-regulated metabolic pathway in carrying out FAO functions but not the autocrine manner of hormone. Further research is proposed to work on downstream elements in the crosstalk.

Acknowledgement

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References

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