北海道大学 大学院農学院 修士論文発表会,2019年2月7日 The effect of co-culture with adipocyte on fiber type commitment of myoblast (脂肪細胞との共培養が筋細胞の筋線維型決定に及ぼす影響)

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1. Introduction

Skeletal muscle and adipose tissues are two major organizations of meat. Skeletal muscle is mainly composed of slow and fast muscle fiber types which are distinct in contractile and metabolic characteristics and are important factors affecting meat quality. However, the process of muscle fiber type commitment is still unclear. Adipose tissue is composed of matured adipocytes, which can secrete various cytokines regarded as adipokines that affect myoblasts growth and development. The aim of this study is to investigate if adipose tissue resident in skeletal muscle influence the process of fiber type commitment of myoblasts through cytokines released from adipocytes.

2. Materials and Methods

C2C12 myoblasts were cultured with conditioned media collected from 3T3-L1 adipocytes differentiated for 10-16 days. The mRNA and protein expression levels of fiber type associated factors, Sema3A or PPAR δ , and myosin heavy chain (MHC) in C2C12 myoblasts were analyzed by real-time RT-qPCR and Western Blotting. Furthermore, myoblasts expressed fast and slow MHC tagged with KusabiraOrange and GFP respectively were cultured with the same conditioned media and the fusion index and ratio of fast/slow myotubes were analyzed.

3. Results and Discussion

There was no significant difference in the mRNA and protein expression levels of Sema3A or PPAR δ between C2C12 myoblasts cultured with conditioned media and control. When C2C12 myoblasts were cultured with conditioned medium of 3T3-L1 adipocyte differentiated for 10 days, the mRNA expression level of fast MHC was significantly decreased (p<0.05) while the protein expression of fast MHC was significantly increased (p<0.05) compared with those in control cells. However, both mRNA and protein expression levels of slow MHC showed no significant difference between them. This result suggested that adipocyte may affect the expression of fast MHC in differentiated myoblasts in the form of endocrine, although the further investigation is needed to elucidate the mechanism.

The fusion index of muscle fiber that predominantly expressed slow MHC was higher when cultured in conditioned media compared with control while the result was opposite in those predominantly expressed fast MHC. This result suggests the possibility that cytokines secreted from adipocytes may promote slow muscle fiber formation.

4. Conclusion

From the current results, there are certain cytokines derived from 3T3-L1 adipocytes that influence the expression of fast MHC in C2C12 myoblasts and have different effects on distinct types of muscle fibers in myotube formation.