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THE ELASTIC STIMULATION MODELS IN MYOBLAST CULTURES REGULATE THE AUTONOMOUS MYOFIBER-TYPE COMMITMENT SYSTEMS

- A novel idea of the regulatory system for cultured meat quality -

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I. INTRODUCTION

Myofiber types have a strong relationship with meat qualities and can be classified into slow- or fast-twitch muscles based on colors, hardnesses, contractile properties, and metabolisms. In general, myofiber type is influenced by neural stimulation patterns, hormone activity, nutrition, and genetic determinations [1]. We currently focus on the key role of satellite cell-derived myoblasts in regulating myofiber types; satellite cells, resident myogenic stem cells, are indispensable for the successful regeneration and hypertrophy of skeletal muscle fibers [2]. In an *in vitro* culture model, myoblasts isolated from muscles of different myofiber types formed myotubes with the original myofiber type based on their localization [3–5]. We also confirmed that satellite cell-derived myoblast cultures isolated from soleus muscle (slow-myofiber abundant) or extensor digitorum longus (EDL; fast-myofiber abundant) formed myotubes with specific myofiber types corresponding to the resident muscle tissue, respectively [6]. Therefore, myoblasts possess the autonomous myofiber-type commitment systems. However, the mechanisms of how myoblasts recognize their origin in slow- or fast-type muscles are unclear. In this study, we assessed whether the physical stimulation model, like stiffnesses (slow-myofiber is soft and fast-myofiber is hard) and exercise patterns that vary by myofiber types, would affect cultured myoblasts.

II. MATERIALS AND METHODS

We prepared “the elastic stimulation model”, “the electric pulse stimulation (EPS) model”, and “the mechanical stretch stimulation (MSS) model” in the myoblast culture system. In this study, we used the myoblast single sub-cultures isolated from 8-week-old male wild-type C57BL/6NJcl (B6) mice EDL and C2C12 mouse myoblasts.

In the elastic stimulation model, myoblasts were cultured on non-toxic treated polyacrylamide gel substrates with 7.5, 13.8, and 67.2 kPa stiffness, according to the previous study [7]. Myoblasts were maintained in a differentiation medium (Dulbecco’s modified Eagle’s medium [DMEM] containing 5% Horse serum, 0.5% gentamicin reagent, and 1% AA-mix) for 72, 120, or 168 h to induce myogenic differentiation into myoblasts and myotubes. In a combination model of elasticity stimulation and EPS, cultured myoblasts on polyacrylamide gel substrates were subjected to EPS (Frequency 1 or 5 Hz; Voltage 40 V) during 70–72 h or 118–120 h after the induction of myogenic differentiation. The EPS stimulation employed C-Dish and C-Pace (IonOptix) and was applied to myoblasts as in the previous study [8]. In the MSS model, differentiated myoblasts on the silicon stretch chambers were elongated as uniaxial extension using Shellpa (Menicon). The low-frequency (LF) loading was set as 10% elongation at a frequency of 0.25 Hz for 1 h, and the high-frequency (HF) loading was 10% elongation at 1 Hz for 1 h. In each culture model, the expression patterns of myosin heavy chain (MyHC) isoforms were measured to evaluate myofiber-type compositions of newly formed myofibers (myotubes). Myofibers are classified into slow-twitch (MyHC type I-positive) and fast-twitch (MyHC type II-positive) fibers, with fast-twitch fibers further subdivided into type IIa, IIx, and IIb-positive fibers. Type IIa represents fast-twitch oxidative fibers; type IIx, intermediate fibers; and type IIb, fast-twitch glycolytic fibers.

The experimental results were statistically analyzed using Student’s *t*-test with Microsoft Excel to analyze differences between the two groups. One-way analysis of variance with post-hoc Tukey-Kramer tests was performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [9]. Data are presented as means ± standard errors of the means (SEMs). Statistical significance was set at a *P*-value of less than 0.05.

III. RESULTS AND DISCUSSION

Initially, we compared the expression patterns of MyHC in myoblasts differentiated on polyacrylamide gels with different stiffness. We could not detect any significant differences in MyHC isoforms until the 120-h culture post-

differentiation. After the 168-h culture, however, the expression level of slow-type MyHC (type I) in cultured myoblasts on 7.5 kPa substrate was higher than other hard substrates, and the fast-type MyHC (type IIb) expression of myoblasts cultured on 67.2 kPa substrate was higher than other substrates (Figure 1). This data suggested that the long-term differentiated myoblasts as myotubes could recognize the stiffness of the culture substrate.
Next, the combination model of substrate elasticity stimulation and EPS induced fast-type MyHC (type IIa, IIx, and IIb) upregulation in myoblasts/myotubes on the culture substrate with 67.2 kPa (Figure 2). This data showed that myoblasts treated with EPS could easily recognize the hard stiffness of the culture substrate to form fast-type myotubes during the short-term differentiation. Then, we also investigated whether the frequency of the MSS model in myoblasts may affect the expression patterns of MyHC isoforms (Figure 3). The LF stimulation induced slow-type MyHC (type I) expression comparing the HF stimulation. This data revealed that slow stretch stimulation is essential for slow-type myotube formation.

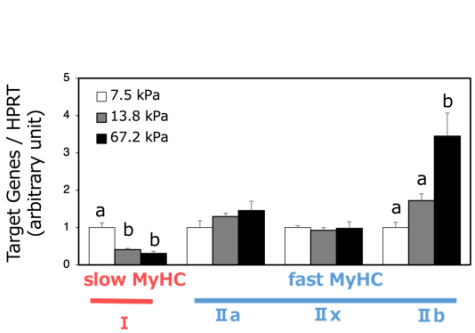


Figure 1
The expression patterns of MyHC in myotubes on polyacrylamide gels 168 h post-differentiation

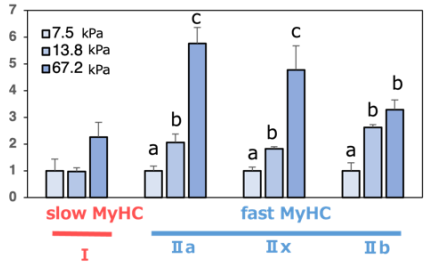


Figure 2
The expression patterns of MyHC isoforms in myoblasts/myotubes treated with the combination of substrate elasticity stimulation and EPS

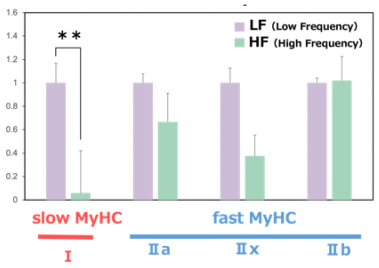


Figure 3
The expression patterns of MyHC isoforms in myoblasts cultured in the MSS model 72 h post-differentiation

IV. CONCLUSION

Taken together, we concluded that the physical stimulation models were key components for cultured myoblasts to acquire the capabilities of autonomous myofiber-type regulation. Cultured myoblasts can form myotubes expressing a specific myofiber type adapting to the physical characteristics like stiffnesses and exercise patterns. These stimulation models may be applied to the control techniques of cultured meat qualities, excluding the preparation of expensive chemicals and reagents.

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