The multi-potent modulator netrin-1 synthesized in satellite cell- derived myoblasts isolated from the fast-twitch muscle may regulate fast-type myotube formation

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Objectives: Resident myogenic stem cells, satellite cells, are indispensable for successful regeneration and hypertrophy of skeletal muscle fibers (myofibers). We currently focus on a novel role of satellite cells in the myofiber-type regulation. Myofibers can be classified into slow- or fast-twitch muscle based on colors, hardnesses, contractile properties and metabolisms, and have strong relationship with meat qualities. Our previous studies showed that satellite cell-derived myoblasts prepared from soleus muscle (slow-myofiber abundant) synthesize and secrete larger amount of semaphorin 3A (Sema3A, a multi-functional protein originally found as a neural chemorepellent) than extensor digitorum longus (EDL; fast-myofiber abundant) at myogenic differentiation phase (Suzuki et al., 2013). The subsequent study demonstrated that Sema3A impacts the formation of slow-twitch myotube after muscle injury (Tatsumi et al., 2017). In contrast to this Sema3A-driven mechanism, there are few knowledges about regulatory mecha- nisms for "fasttwitch myotube commitment" by a secretory protein(s) from satellite cell-derived myoblasts. In this study, we hy- pothesized the function of the multi-potent modulator netrin families (netrin-1, 3 and 4) that may contribute to fast-twitch myotube formation since their physiological significances are known to compete with Sema3A in neurogenesis and osteogenesis.

Materials and Methods: To evaluate our hypothesis, we examined whether netrins impact fast-twitch myotube generation using cell culture system. First, the expression profiles of netrin subtypes and their cell membrane receptors in primary cultures of satellite cells. Next, the knockdown treatment of netrin by specific siRNAs transfection with RNA interference technique in differentiated satellite cell-derived myoblasts. Furthermore, we performed the additional experiments with recombinant netrin.

Results and Discussion: We revealed that netrin-1 was significantly up-regulated like a Sema3A expression pattern during myogenic differentiation phase. Subsequently, we compared the expression levels of netrin subtypes and receptors between soleus- and EDL- derived satellite cells; there were no differences in expression levels of netrin-3, 4 and netrin's cell-membrane receptors (neogenin, BOC, CDO, Unc5B and 5C), while satellite cells from EDL muscle expressed higher levels of netrin-1 than those from soleus. Ne- trin-1 knockdown inhibit myotubes formation by significantly diminishing the expression level of fast-type myosin heavy chain (especially in type IIb). Exogenous netrin-1 not only up-regulated the fast-twitch markers, but also downregulated the slow-twitch marker. These findings suggest that netrin-1 abundantly synthesized in EDL-derived satellite cells would act as a key modulator to promote myofiber-type commitment of fast muscles (Suzuki et al., 2021). This work was supported by JSPS KAKENHI and the grant fund from the Ito Foundation.

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