The IL-33-ST2 Signaling in Fibro-adipogenic Progenitors Attenuates Immobilization-induced Muscle Atrophy

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INTRODUCTION: In principle, muscle atrophy is a reversible condition and could be fully reversed; however, the excellent regenerative and adaptive capacity of skeletal muscle is known to weaken with age. Disuse-induced skeletal muscle atrophy is a cause of severe disability and frailty in the elderly, and it is clinically important to develop a treatment to ameliorate this undesirable condition in the rapidly aging society. In skeletal muscle, there are at least two distinct types of stem cells; muscle satellite cells (SCs) and fibro-adipogenic progenitors (FAPs). FAPs are considered to be the source cells of various skeletal muscle conditions, such as fatty infiltration, heterotopic ossification, and fibrosis. Recent studies also indicate that FAPs are one of the primary regulators of the SCs and muscle fiber function and thereby play a critical role in maintaining the skeletal muscle homeostasis and adaptability. However, their involvement in muscle atrophy is poorly understood. In this study, we aimed to understand the potential involvement of FAPs in muscle atrophy and found that the autocrine IL-33-ST2 signaling in FAPs attenuates immobilization-induced muscle atrophy in mice.
METHODS: Young (2-3 months), adult (12-14 months), and aged (20-22 months) mice were used for analysis. Muscle atrophy was induced by immobilizing the hindlimbs with a steel wire. FAPs were isolated from hindlimbs on days 0, 3, and 14 after immobilization for transcriptome analysis. Differentially expressed genes with more than 2-fold change in both day 3 and day 14 samples compared to the baselines (day 0 samples) were extracted. The expression of ST2 and IL-33 in FAPs was evaluated by flow cytometry and immunostaining, respectively. To examine the role of the IL-33-ST2 signaling in vivo, recombinant IL-33 or soluble ST2 (sST2), which functions as a decoy receptor for IL-33, were administrated intraperitoneally twice a week during the 2-week immobilization period. After 2-week immobilization, the tibialis anterior muscles were harvested and the cross-sectional area of muscle fibers was evaluated. To determine the target cell of IL-33, FAPs and muscle fiber cells were incubated with recombinant IL-33 for 1 h and stained for NF-κB, one of the intracellular molecules located downstream of ST2. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Keio University School of Medicine.
RESULTS: The number of FAPs increased with the progression of muscle atrophy after immobilization in all age-groups. Transcriptome analysis of FAPs collected before and after immobilization revealed that the transcripts for *Il33* and *Il1rl1*, which encodes the IL-33 receptor ST2, are transiently induced in young mice and, to a lesser extent, in aged mice (Figure 1). The number of FAPs positive for ST2 increased after immobilization in young mice. The number of FAPs was also upregulated after immobilization in aged mice, but the difference from the baseline did not reach statistical significance. Immunostaining showed a significant increase in the number of FAPs expressing IL-33 (Figure 2). Administration of recombinant IL-33 suppressed immobilization-induced muscle atrophy in aged mice but not in young mice (Figure 3). IL-33 induced the nuclear translocation of NF-κB in FAPs but not in muscle fiber cells.
DISCUSSION: Our data indicate that the autocrine IL-33-ST2 signaling in FAPs plays a protective role against immobilization-induced muscle atrophy in mice. Furthermore, given that administration of IL-33 was effective in aged mice but not in young mice, it was assumed that the IL-33-ST2 signaling was defective and suppressed in aged mice. Because FAPs, but not muscle fiber cells, responded to IL-33, it is likely that IL-33-activated FAPs produce secretory factor(s) that act on muscle fiber cells and alleviate muscle atrophy. These results suggest that the IL-33-ST2 signaling is a potential therapeutic target for the treatment of disuse muscle atrophy in the elderly.
SIGNIFICANCE: Our study reveals previously unknown role of the IL-33-ST2 signaling in FAPs in protecting against immobilization-induced muscle atrophy and suggests that the IL-33-ST2 signaling is a novel therapeutic target for muscle atrophy.
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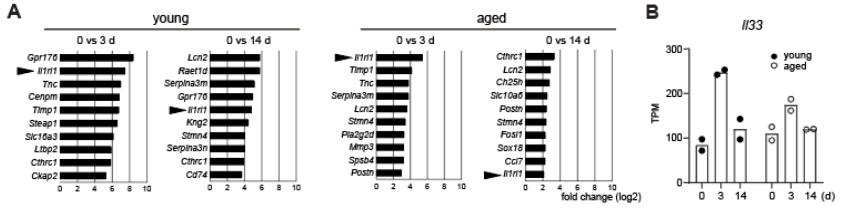

Figure 1. Expression of *Il33* and *Il1rl1* transcripts in FAPs is induced after immobilization. *A*, lists of the top 10 differentially expressed genes that showed the greatest increase from the baseline controls on day 3 and day 14 of hindlimb immobilization. ***B***, the transcripts per million values of *Il33* in FAPs of young and aged mice on day 0, day 3, and day 14 of immobilization. *N* = 2.

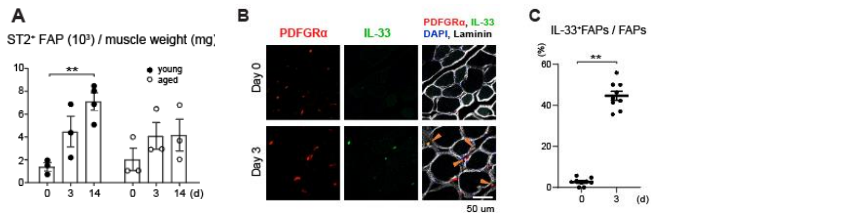

Figure 2. ST2-positive FAPs and IL-33-positive FAPs are induced upon immobilization. *A*, the number of ST2-positive FAPs per muscle weight in the tibialis anterior muscles collected on day 0, day 3, and day 14 of immobilization. *N* = 3. *B*, sections of the tibialis anterior muscle of young mice. Specimens were collected on day 0 and day 3 of immobilization. *C*. Ratio of FAP cells positive for ST2 on day 0 and day 3 of immobilization. Three random sections from each specimen (three mice) were evaluated. *, P < 0.05; **, P < 0.01. Bar, 50 μm.

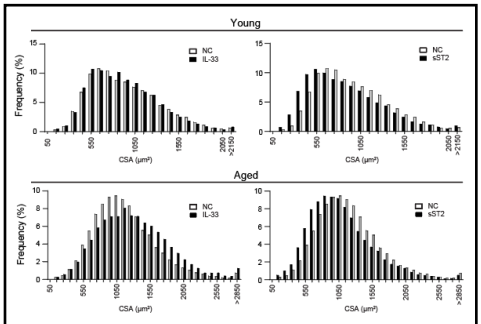

Figure 3. The IL-33-ST2 signaling suppresses immobilization-induced muscle atrophy. Frequency distribution of cross-sectional area of myofibers of tibialis anterior muscles collected from IL-33-treated and sST2-treated young (upper panels) and aged (lower panels) mice. *N* = 4 mice. NC, vehicle-treated negative control.