Responses of circulating microRNAs to unloading-associated atrophy and reloading-induced regrowth of mouse hindlimb skeletal muscle

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MicroRNAs (miRNAs) are small (~22 nucleotides) non-coding RNAs, which play important roles in the regulation of gene expression at the post-transcriptional level through mechanisms such as translational inhibition or mRNA degradation. There are many evidences regarding the roles and regulation of miRNAs in skeletal muscle plasticity (Aoi *et al*., 2014; Goto *et al*., 2013). In addition, many cells secrete miRNA-loaded exosomes into circulation. In fact, miRNAs are confirmed in exosomes in serum and plasma (Baggish *et al*., 2011). Recently, several circulating miRNAs have been established as biomarkers for various diseases, such as cancer, liver injury, and heart failure. Skeletal muscle-specific miRNAs, which highly expressed in skeletal muscle cells, has been confirmed in plasma and serum of patients with neuromuscular disorders (Toivonen *et al*., 2014). However, it is still unclear whether circulating miRNA(s) could be a biomarker for skeletal muscle plasticity or not. In the present study, we investigated the responses of plasma miRNAs to hindlimb unloading-associated atrophy and reloading-associated regrowth of skeletal muscle.

All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Bethesda, MD, USA) and were approved by the Animal Use Committee of Toyohashi SOZO University. Eleven-week-old male mice (C57BL/6J) were used. All mice were housed in a vivarium room with 12:12-h light:dark cycle and ~23°C and ~50% temperature and humidity, respectively. Solid food and water were provided *at libitum*. Mice were randomly divided in to control and unloaded groups. Mice of unloaded groups were subjected to 2-week hindlimb suspension followed by 2-week ambulation recovery. Blood and soleus muscles were sampled before hindlimb suspension (Pre), immediately after the suspension (R0), and 2-week recovery (R2). Statistical significance was evaluated by one-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey-Kramer test. The significance level was accepted at $P<0.05$.

Soleus weight relative to body weight was decreased following 2-week hindlimb suspension, and was increased following reloading ($P<0.05$). Microarray analyses for miRNAs revealed that 65 miRNAs were increased in plasma by 2 weeks of the suspension, and decreased following 2-week recovery. Large increases and decreases in circulating miR-135a-2, miR-491, miR-5115 were observed following unloading and reloading, respectively. On the other hand, five miRNAs including miR-7a and miR-208a, were decreased in plasma by the unloading, and were increased by the reloading. Real-time reverse transcription-polymerase chain reaction (RT-PCR) analyses also showed that the level of plasma miR-206, a skeletal muscle-specific miRNA, was increased by unloading and decreased by reloading ($P<0.05$). Therefore, some miRNAs in plasma could be a biomarker for the evaluation of the skeletal muscle conditions in response to various extracellular stimuli.

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