Mechanical stretch inhibits myoblast-to-adipocyte trans-differentiation through Wnt signaling

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Introduction: Myogenic stem cells such as C2C12 cells in adult skeletal muscle (satellite cells) maintain multi-differentiation potential and are able to differentiate into non-muscle cells including osteoblasts and adipocytes. This transition between one differentiated lineage to another is called trans-differentiation. The signaling mechanisms that are responsible for trans-differentiation are poorly understood. The normal development of skeletal muscle is controlled by myogenic regulatory factors (MRFs), such as myogenin and MyoD. On the other hand, differentiation of adipocytes appears to be controlled by peroxisome proliferator activating receptor (PPAR-γ) and the C/EBP families of transcription factors. The balance between these myogenic and adipogenic transcriptional factors is related to commitment of cell differentiation.

We have previously shown that mechanical stretch regulates expression of transcriptional factors influencing cell differentiation. Application of cyclic mechanical stretch down-regulates expression of MyoD and MNF-α in C2C12. Based on these findings, we hypothesized that mechanical stretch affects the balance between myogenic and adipogenic transcriptional regulatory factors.

In this study, we investigated the effect of mechanical stretch on C2C12 muscle satellite cells during induced adipogenesis. We show that mechanical stretch inhibits C2C12 cell adipogenic differentiation through activated Wnt signaling.

Materials and Methods: Induction of adipogenesis For adipogenic differentiation, C2C12 cells were cultured in an adipogenic induction medium (4.5 g/l glucose, 10% FBS, 1 mM dexamethasone, 0.5 mM isobutylmethylxanthine, 10 mg/ml insulin in DMEM). After 7 days, the cells were used for further analyses.

Mechanical stretch of C2C12

Cyclic mechanical stretch was applied to cultured C2C12 cells in vitro using a BioFlex strain unit (Flexcell International Co., Hillsborough, NC). A vacuum (17 kPa) was applied at a frequency of 6 cycles/min (3-sec on-time, 7-sec off-time) from the base of the plate. Cells cultured on the same type of plates without stretch were served as control.

Results: Mechanical stretch inhibits adipogenic differentiation in C2C12 cells.

C2C12 cells were cultured with cyclic mechanical stretch during induced-adipogenic differentiation. Mechanical stretch maintained C2C12 cells in spindle shape while cells without stretch treatment acquired a round shape during adipogenic differentiation (Fig 1). Furthermore, the expression of PPAR-γ mRNA was decreased by stretch in adipogenic induction medium.

Mechanical stretch inhibits adipogenesis via the Wnt signaling.

We compared the expression of Wnt10b, which is known to stabilize cytosolic β-catenin in Wnt signaling, between stretched and unstretched cells under adipogenic conditions. Wnt10b mRNA was increased 2.5-fold in mechanical stretched cells compared with sister cultures without stretch. To confirm that the profound effects of mechanical stretch were indeed attributable to Wnt signaling, we added sFRP-2, a soluble receptor of Wnt10b, into the culture. Addition of sFRP-2 completely blocked stretch-induced expression of PPAR-γ mRNA (Fig 2).

Discussion: This study demonstrates that differentiation from myoblasts to adipocytes is inhibited by cyclic mechanical stretch. Blocking Wnt signaling by a soluble receptor of Wnt, sFRP-2, abolished the mechanical stretch-induced inhibition of adipogenesis. These results suggest that mechanical stretch inhibits the myoblast-to-adipocyte differentiation via the Wnt signaling.

Our findings may be relevant to understanding the pathogenesis of obesity and skeletal muscle diseases. Understanding the signaling pathways that mediate trans-differentiation between myoblasts and adipocytes should be of relevance to the development of therapeutics for the treatment of obesity and impaired metabolism in sedentary population.

Our results might also provide new insights into the relationships between exercise and body composition. It is generally known that exercise decreases fat mass due to increased energy expenditure during muscle contraction. However, our current data suggest that exercise-associated mechanical stimuli may block pathogenic trans-differentiation of myogenic satellite cells into pre-adipocytes. Our results suggest that mechanical stretch may block the myoblast-to-adipocyte differentiation process.

References:

Figure 1. Mechanical stretch inhibits adipogenic differentiation of C2C12 cells. Unstretched cells in adipogenic medium assume the rounded shape of adipocytes (A) while stretched cells remain spindle-shaped (B). Stretch reduced expression of PPARγ mRNA (C, D).

Figure 2. Mechanical stretch-induced inhibition of adipogenesis requires Wnt signaling. C2C12 cells cultured in adipogenic induction medium with sFRP2 to inhibit Wnt signaling expressed PPARγ mRNA in the manner of unstretched cells.