Wnt7a-Releasing Hydrogel Inhibits Adipogenesis of Skeletal Muscle Mesenchymal Stromal Cells

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Introduction: Rotator cuff (RC) tendon tears are common upper extremity injury that results in persistent atrophy and fatty infiltration of the associated muscles. Previously, we engineered Wnt7a-releasing hydrogel for promoting muscle regeneration through localized and controlled administration of Wnt7a.[1] Hydrogel-released Wnt7a improves muscle regeneration by promoting myofiber hypertrophy and satellite cell expansion through non-canonical pathways,[2,3] suggesting its potential clinical application for treating RC muscle degeneration. However, the role of Wnt7a in inhibiting adipogenesis of fibro-adipogenic progenitors (FAPs), the primary cellular culprit of RC muscle fatty infiltration, remains unknown. Thus, the objective of this study was to determine the effects of Wnt7a on modulating adipogenesis of murine FAPs and to confirm that hydrogel-released Wnt7a retains its bioactivity in suppressing FAPs adipogenesis.

Methods: ***FAPs Isolation.*** Primary FAPs were isolated from hind limb muscles of 3-5-week-old C57B6/J mice through magnetic-activated cell sorting. FAPs were obtained through negative cell selection (CD31-, CD45-, α7 integrin-) followed by positive cell selection (Sca1+). ***FAPs Expansion and Differentiation.*** FAPs were expanded to 80% confluency for 4 days on laminin (10 μg/mL) and collagen I (5 μg/mL) coated plates. To induce adipogenic or fibrogenic differentiation, FAPs were further cultured in Adipogenic Media or Fibrogenic Media, respectively, for additional 4 days. Carrier-free human recombinant Wnt7a reconstituted in sterile water (200 ng/ml unless indicated in the figures) was added at the time of the ADM switch. ***Bioactivity Assessment of Wnt7a-releasing Hydrogel.*** Wnt7a-releasing hydrogels were synthesized by encapsulating Wnt7a in 20 kDa 4-arm polyethylene glycol (PEG)-norbornene crosslinked using protease degradable VPM peptide. To determine if the released Wnt7a retains its bioactivity, the hydrogel was placed in the media containing FAPs on day 1. FAPs were allowed to spontaneously differentiate in growth media, media containing Wnt7a (200 ng/ml), and media containing a Wnt7a-releasing hydrogel (200 ng/ml) for 7 days. ***Staining & Analyses.*** Cells were fixed and labeled. Image analysis was performed in an automated manner using ImageJ software. ***Statistical Analyses.*** One-way ANOVA and Tukey's multiple comparisons analysis were used for Wnt7a dose-response and Wnt7a hydrogel bioactivity assays. Unpaired t-test was used to perilipin and PPAR-γ analyses. Significance was set at $p<0.05$.

Results: Freshly isolated FAPs expressed PDGFR-α (18 hr; **Figure 1a**). Furthermore, FAPs successfully differentiated into αSMA+ myofibroblasts and Oil Red O/perilipin+ adipocytes when cultured in fibrogenic media and adipogenic media, respectively (**Figure 1a**). In both growth media and ADM conditions, FAPs treated with increasing concentration of Wnt7a exhibited decreasing levels of adipogenesis (% Oil Red O+ cells) in a dose-dependent manner (**Figure 1b, c**). Through this assay, we observed that 200 ng/ml Wnt7a significantly reduced FAPs adipogenesis ($p=0.0478$; **Figure 1b, c**), and thus this concentration was used in the subsequent experiments. To further confirm the effect of Wnt7a in suppressing FAPs adipogenesis, we next evaluated changes in the expressions of perilipin-1 and peroxisome proliferator-activated receptor gamma (PPAR-γ) with Wnt7a treatment. FAPs treated with Wnt7a exhibited significantly decreased perilipin ($p=0.0207$) and PPAR-γ ($p=0.0013$) staining (**Figure 1d, e**). To determine if Wnt7a delivered using the synthetic hydrogel system maintains its bioactivity, we treated differentiating FAPs with hydrogel-free Wnt7a and Wnt7a-releasing hydrogel (**Figure 1f**). FAPs cultured in the absence of Wnt7a gave rise to Oil Red O+ adipocytes, but both hydrogel-free Wnt7a and Wnt7a-releasing hydrogel treatment significantly inhibited FAPs adipogenesis at a similar level ($p<0.001$; **Figure 1f, g**).

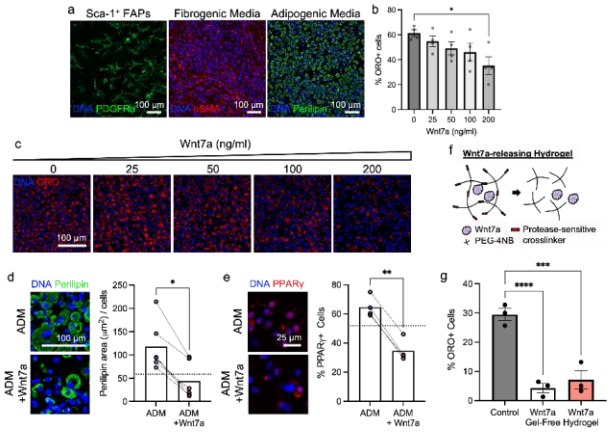

Figure 1. a) Isolated PDGFR-α+ FAPs differentiate into myofibroblast and adipocyte. **b,c)** Wnt7a inhibits adipogenesis in a dose-dependent manner. Oil red O staining. **d)** Wnt7a reduces perilipin+ adipocytes. **e)** Wnt7a reduces PPAR-γ activity. Dotted lines: mean value of FAPs spontaneously differentiated in growth medium. **f,g)** Protease-sensitive Wnt7a releasing hydrogel maintains bioactivity in reducing FAPs adipogenesis. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions: In this study, the inhibitory effect of Wnt7a on FAPs adipogenesis was confirmed. Wnt7a released from the engineered synthetic hydrogel also maintained its bioactivity in vitro. Collectively, the hydrogel system is a suitable biomaterial platform for administering Wnt7a in a controlled manner.

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References: [1] Han+ Acta Biomater 2019; [2] von Maltzahn+ Nat Cell Bio 2011; [3] Le Grand+ Cell Stem Cell 2009