Role of beta-catenin in the development of skeletal myocytes

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Abstract

Skeletal muscle wasting is a prominent symptom in a wide range of human conditions, some of which include muscular dystrophy, sarcopenia, cancer, sepsis, and HIV-infection. Cell-based therapies using various stem cell types have great potential for treating and reversing skeletal muscle wasting, however there remains the obstacle of improving the efficacy of stem cell differentiation into skeletal myocytes. The myogenic regulatory factors (MRFs) are the main muscle-specific transcription factors that control muscle differentiation, which are in turn regulated by signal transduction pathways. Beta-catenin is a main activator of one such pathway, and it affects the specification and pattern formation in the early vertebrate embryo. Establishing the connections between beta-catenin and other signaling pathways could improve our knowledge of skeletal myocyte development from stem cell differentiation, and lead to the development of cell-replacement therapies. To determine the influence of beta-catenin on skeletal myogenesis we used pluripotent stem cell lines, one containing a functional knockout of beta-catenin and the other serving as a control. With the small molecules bexarotene (BEX) and retinoic acid (RA) being utilized as differentiation inducers, the efficacies of the skeletal myocyte development were determined by quantitative analysis of skeletal muscle differentiation, which are in turn regulated by signal transduction pathways.

Background

The canonical Wnt signaling pathway. In the absence of Wnt, beta-catenin is present in the cytoplasm in small quantities because it is degraded as a result of phosphorylation by the Axin/APC/GSK3beta complex. If Wnt is present, the Axin/APC/GSK3beta complex is inactivated, allowing beta-catenin to accumulate in the cytoplasm and to enter the nucleus. Beta-catenin will then interact with the transcription factor of the TCF family, upon entering the nucleus, to activate gene transcription.

Results

Figure 1. Beta-catenin is important for skeletal myocyte development. (A) Cells were allowed to aggregate for four days in the presence of DMSO or DMSO and either retinoic acid (RA) or bexarotene (BEX). They were then cultured for an additional five days without any treatment, and were stained for Myosin Heavy Chain (MyHC) and Myogenic Differentiation 1 (MyoD). (B) Quantification of the differentiated skeletal myocytes stained for MyHC. (C) Quantification of the differentiated skeletal myocytes stained for MyoD. For both figures 1B and 1C, the quantifications for the dominant-negative engrafted beta-catenin cells (β-engl) are shown adjacent to that of the associated treatment control cells.

Figure 2. Myogenin protein expression during skeletal myogenesis. (A) Quantification of Myogenin expression for dominant-negative beta-catenin cells. (B) Western blot of day 9 control cells and dominant-negative beta-catenin cells. Day 9 control cells were used as a control and β-Tubulin was used as a loading control. Cells were aggregated for four days in the presence of DMSO or DMSO and retinoic acid or bexarotene, and then were maintained for five additional days before being harvested on day 9.

Figure 3. Pax3 mRNA levels enhanced by RA and BEX in dominant-negative beta-catenin cells during skeletal myogenesis. Real-Time Q-PCR quantification was completed to detect transcription of mRNA of Pax3 using GAPDH as the internal control. Cells were aggregated for 4 days with 1% DMSO or 1% DMSO and either retinoic acid (RA) or bexarotene (BEX), and then were stained for Myosin Heavy Chain (MyHC), MyoD and Hoescht.

Conclusions

- Beta-catenin is important for skeletal muscle development.

References

