Role of beta-catenin in the development of skeletal myocytes

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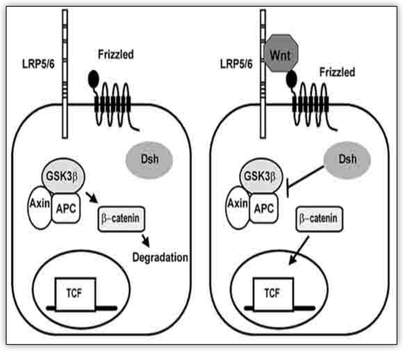
Abstract

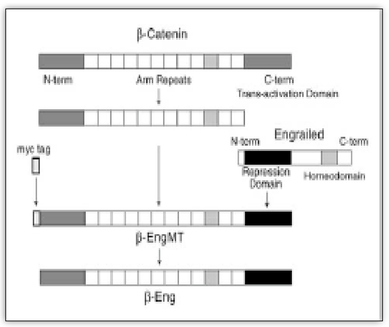
Skeletal muscle wasting is a prominent symptom in a wide range of human conditions, some of which include muscular dystrophy, sarcopenia, cancer, sepsis, and HIV-infection. Cell-based therapies using various stem cell types have great potential for treating and reversing skeletal muscle wasting, however 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 skeletal 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 skeletal myogenesis constituents 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 in stem cell 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 specific markers. Our data suggests that beta-catenin plays an essential role in skeletal myogenesis, and that small molecular inducers, such as BEX and RA, depend on proper beta-catenin activity to enhance the efficacy of differentiation.

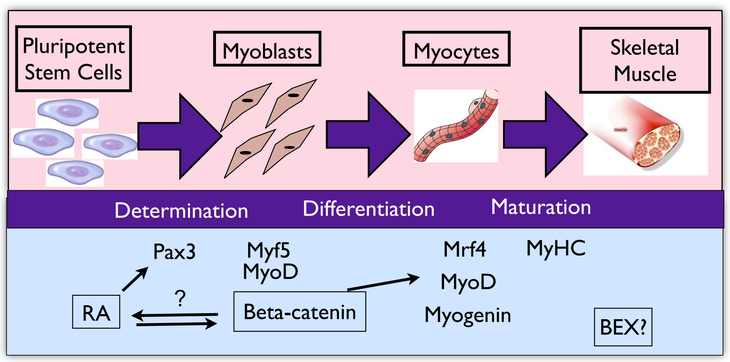
Background



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.**

Dominant negative beta-catenin engrailed (β-eng) cell line. **β-eng is a chimeric protein in which the activation domain of beta-catenin is replaced with a transcriptional repression domain of engrailed, preventing the regular function of beta-catenin. For the purposes of this project, a dominant-negative beta-catenin cell line (β-eng) was used in conjunction with a control vector cell line to determine if beta-catenin is needed for skeletal myocyte development.**

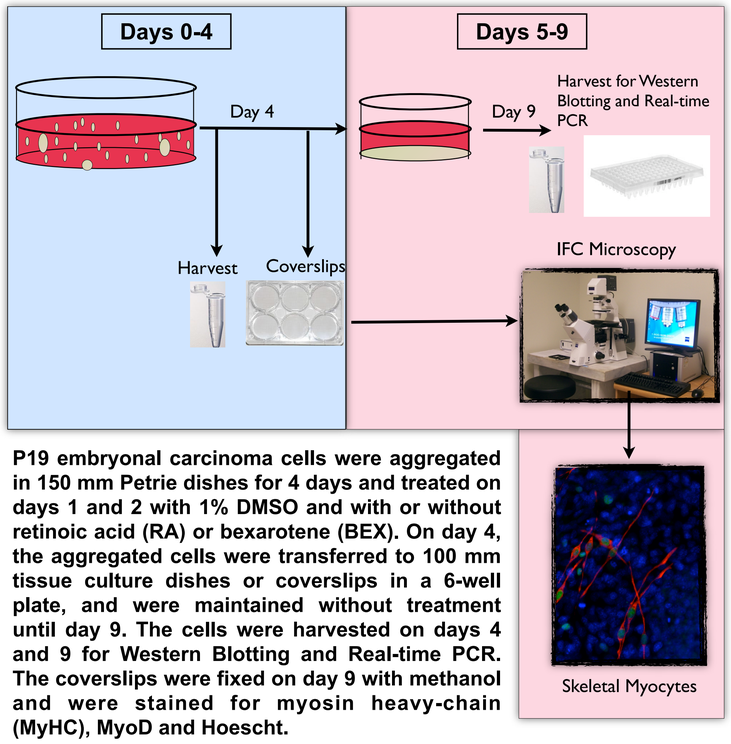




Hypothesis

➡ **Beta-catenin is important for myogenic conversion enhanced by the small molecular inducers retinoic acid and bexarotene.**

Methodology



P19 embryonal carcinoma cells were aggregated in 150 mm Petrie dishes for 4 days and treated on days 1 and 2 with 1% DMSO and with or without retinoic acid (RA) or bexarotene (BEX). On day 4, the aggregated cells were transferred to 100 mm tissue culture dishes or coverslips in a 6-well plate, and were maintained without treatment until day 9. The cells were harvested on days 4 and 9 for Western Blotting and Real-time PCR. The coverslips were fixed on day 9 with methanol and were stained for myosin heavy-chain (MyHC), MyoD and Hoescht.

Results

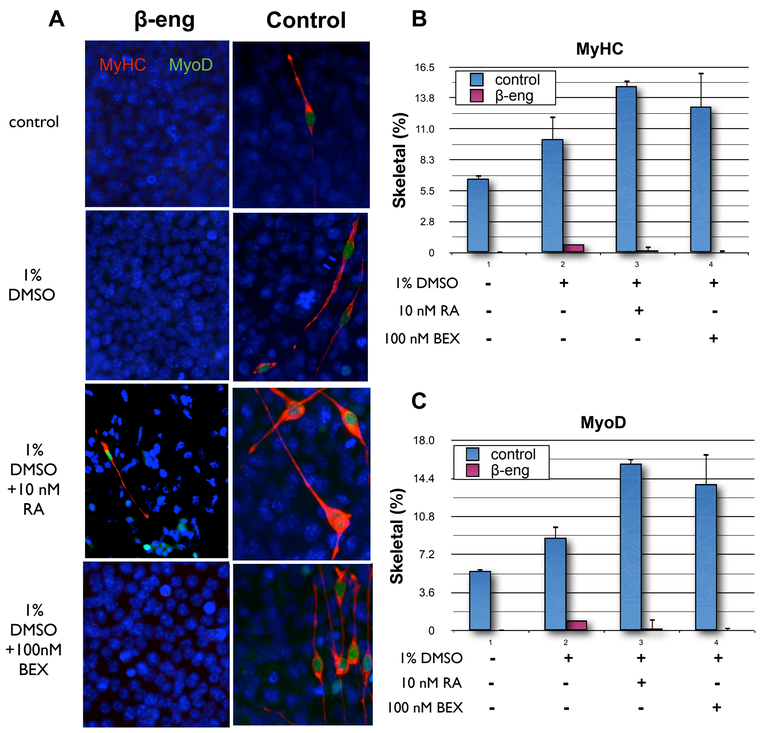


Figure 1. ***Beta-catenin is important for skeletal myocyte development.*** **(A) Cells were allowed to aggregate for four days in the presence of DMSO or DMSO and either retinoic acid (RA) or bexarotene (BEX). They were then cultured for an additional five days without any treatment, and were stained for Myosin Heavy Chain (MyHC) and Myogenic Differentiation 1 (MyoD) on day 9. (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 engrailed beta-catenin cells (β-eng) are shown adjacent to that of the associated treatment control cells.**

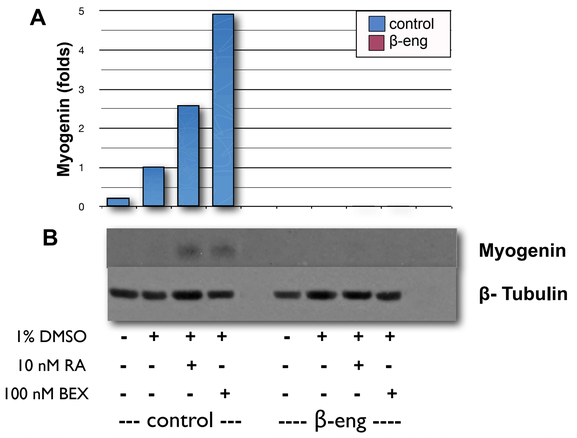


Figure 2. ***Myogenin protein expression during skeletal myogenesis.*** **(A) Quantification of Myogenin expression for dominant-negative beta-catenin cells. (B) Western blot of day 9 control cells and dominant negative beta-catenin cells. Day 9 1% DMSO control cells were used as a control and β-Tubulin 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.**

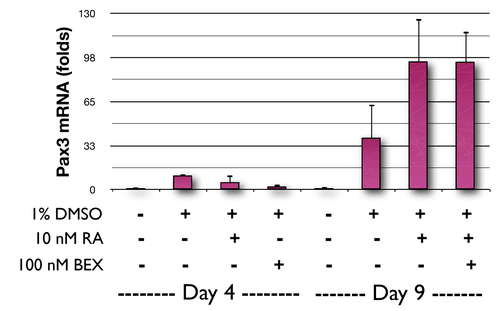


Figure 3. ***Pax3 mRNA levels enhanced by RA and BEX in dominant-negative beta-catenin cells during skeletal myogenesis.*** **Real-Time (Q-PCR) quantification was completed to detect transcription of mRNA of Pax3 using GAPDH as the internal control. Cells were aggregated for 4 days with 1% DMSO or 1% DMSO and either retinoic acid (RA) or bexarotene (BEX), and harvested on days 4 and 9. Quantification was carried out to detect transcription of mRNA for Pax3 and MyoD with GAPDH as internal control. The error bars represent the standard deviation of Pax3 mRNA levels for one experiment.**

Conclusions

➡ **Beta-catenin is important for skeletal muscle development.**
As presented by the efficacy of myogenic conversion, by the myogenin protein expression levels, and by the Pax3 mRNA levels, the dominant-negative beta-catenin cells demonstrated lower levels (if any) of skeletal myocyte development when compared with the control cells.

➡ **Small molecular inducers depend on proper beta-catenin function to enhance skeletal myocyte development.**
Bexarotene and retinoic acid both increased the efficiency of skeletal myocyte differentiation in the control cells. However, no enhancement was observed in the dominant-negative beta-catenin cells, suggesting that the small molecular inducers cannot bypass beta-catenin.

Future work

Determine the expression of the other myogenic regulatory factors (MRFs)

➡ Establish the expression of Myf5, Meox1, Meox2, MyoD, and Gli2 with Western Blotting and Real-time PCR.

Use small interfering RNA (siRNA), or silencing RNA, and embryonal stem (ES) cells

➡ Determine the role of beta-catenin using silencing RNA, rather than the engrailed approach. This will determine whether beta-catenin is essential for skeletal myocyte development.

➡ Establish a more physiological model to study the role of beta-catenin in skeletal myogenesis and its implications in cell-based therapies with embryonal stem cells, compared to that established using embryonal carcinoma (EC) cells.

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