Activation of Wnt Planar Cell Polarity (PCP) signaling promotes growth plate column formation in vitro

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BACKGROUND:

Growth plate regeneration offers the potential to restore longitudinal bone growth in pediatric patients, but the success of this approach depends upon recapitulating the columnar architecture of the native growth plate. Although the identity of some of the critical factors regulating growth plate column formation have been suggested through studies of transgenic mice, previous investigators have no succeeded in regenerating columnar growth plate tissue in vivo. The objective of this study was to test the hypothesis that activation of the Wnt Planar Cell Polarity (PCP) signaling pathway in growth plate chondrocytes cultured as three-dimensional cell pellets would promote morphogenesis of columnar growth plate architecture in vitro.

METHODS:

Epiphyseal chondrocytes were isolated from distal femoral epiphyses of 4-day old Sprague-Dawley rats, transfected with plasmid DNA expressing Fzd7 (EE epitope tag), a deletion mutant of Fzd7 lacking the Wnt binding domain, Vangl2 (HA epitope tag), or Ror2 (FLAG epitope tag) and cultured as three-dimensional cell pellets with recombinant Wnt5a or Wnt5b for 21 days in 1% FBS DMEM:F12 media. Pellets were sectioned and stained with toluidine blue. Cellular morphology and columnar architecture were evaluated using a custom image analysis program that calculated histomorphometric measurements for which column requirements included flattened cellular morphology (aspect ratio ≥ 1.25), alignment of adjacent cells (lines connecting adjacent cells within 20 degrees of 180), and close proximity of adjacent cells (≤ 20 pixels). Novel column index values were calculated as the proportion of cells in each field participating in columns of ≥ 3 cells multiplied by the average column length in pixels. Output measures were compared using one-way ANOVA for multiple treatment groups, or Student’s t-test for pairwise comparisons with significance level of α = 0.05. Immunoprecipitation-immunoblotting experiments were performed according to standard protocols.

RESULTS:

Activation of Wnt PCP signaling significantly enhanced columnar morphogenesis in the pellet culture model compared to untreated controls (ANOVA P = 0.01). Stacks of flattened chondrocytes resembling the proliferative zone cells of the native growth plate were apparent in pellets transfected with Fzd7, Ror2, and treated with Wnt5a (Figure 1). Wnt5a treatment increased column index values in all treatment groups except Fzd7 Vangl2 co-transfection, while overexpression of Vangl2 was largely inhibitory (Figure 2). Immunoprecipitation-immunoblotting experiments demonstrated that Wnt5a resulted in not only association of Ror2 and Vangl2, but also association of Fzd7 and Ror2, as well as Fzd7 and Vangl2 (Figure 3).

DISCUSSION:

Activation of the Wnt PCP signaling pathway through overexpression of Fzd7 and Ror2, and addition of Wnt5a promoted the initiation of columnar morphogenesis of growth plate chondrocytes in vitro. Wnt5a treatment also resulted not only in association of Vangl2 and Ror2, but also Fzd7 with Ror2 and Fzd7 with Vangl2, suggesting that Fzd7 participates in a trimeric receptor complex containing Ror2 and Vangl2 which modulates Wnt5a signal transduction and Wnt PCP signaling.

SIGNIFICANCE:

These results highlight the importance of activating the Wnt PCP signaling pathway in the development of columnar morphogenesis of growth plate tissue in vitro, and hence represent and important step toward the engineering of functional growth plate tissue in vivo.