STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: Response of pediatric lung mesenchymal stromal cells (MSCs) to Wnt pathway agonists and antagonists

Background: The Wnt pathway is crucial in development and repair throughout the human body. Previously, activation of this pathway has been thought to lead to an increase in cellular proliferation. However, recent evidence has found Wnt pathway activation may lead to decreased proliferation and increased differentiation in Mesenchymal Stromal Cells (MSCs). MSCs have recently become the focus of several clinical trials due to their immune modulation role and role in fibrosis, especially in Bronchopulmonary Dysplasia, a fibrotic chronic lung disease of premature infants. The actions of Wnt pathway activation have yet to be fully understood in human MSCs, especially in the lung.

Objective: The present study aims to describe the actions of Wnt pathway agonists and antagonists in pediatric lung MSCs by adding four different doses each of lithium chloride (LiCl; a known Wnt agonist), Secreted Frizzled Related Protein 1 (SFRP1; Wnt antagonist), and Insulin like Growth Factor Binding Protein 5 (IGFBP5; Wnt agonist) to primary pediatric human lung MSCs and measuring the effect on cellular proliferation, cell phenotype, and gene expression.

Methods: MSCs from 5 donors (DP01, DP03, DP04, DP05, DP06) aged 1 day old to 8 years old were acquired from previously frozen vials of sorted early passage pediatric lung donor cells as part of the LungMap program. MSCs were seeded at 20,000 cells per well in 12-well dishes with growth media (αMEM +10% Fetal Bovine Serum and 2% antibiotics-antimycotics). After 24 hours, media was replaced with growth media containing respective treatment doses of the following compounds: LiCl (160, 40, 10, and 2.5 mM), SFRP1 (250, 50, 10, and 2 ng/mL), and IGFBP5 (100, 20, 4, and 0.8 nM). Each treatment was run in duplicate. After 72 hours, cells from each well were imaged, harvested, and counted. RNA was extracted, quantified, and analyzed by qPCR. In addition, 6 well dishes were seeded with 50,000 cells/well and treated in duplicate with 10 and 2.5 mM LiCl. Cells were harvested, stained with an MSC antibody panel, and run on flow cytometry. Wnt pathway gene expression was run with previously diluted cDNA using primers for Wnt2, Fzd7, LRP6, and Axin2 by qPCR.

Results: In each donor, treatment of LiCl resulted in reduced cell expansion and a phenotypic change towards a wider and more blunted cell, resembling MSCs at a higher cell passage number. The effects were dose dependent, with the highest dose of LiCl demonstrating the greatest decrease in expansion. While addition of either SFRP1 or IGFBP5 led to varied responses in each donor, general trends were observed. For SFRP1, high concentrations led to a general increase in fold expansion, while fold expansion was decreased when the two lowest concentrations were added. This supports the biphasic activity of SFRP1 as an agonist at low concentrations and an antagonist of the Wnt pathway at high concentrations. Increasing IGFBP5 levels led to a plateau in the fold expansion after an initial decline at low concentrations. For DP05, LiCl concentrations were inversely proportional to TGFBIGH3 expression ($p=0.0008$). Wnt2 and Fzd7 yielded the highest fold expression relative to PPIA and had the highest relative expression in DP03 and DP01 compared to other donors.

Conclusion: Addition of LiCl to pediatric lung MSCs leads to a decrease in expansion in a dose-dependent manner across various ages. Further studies are needed to elucidate the donor-dependent roles of SFRP1 and IGFBP5 found in this study in lung MSCs. Future directions should also explore the role of Wnt activation in BPD lungs based upon treatment with the Wnt agonists and antagonists.