

Canonical/beta-catenin Wnt pathway activation improves retinal pigmented epithelium derivation from human embryonic stem cells.

Journal: Invest Ophthalmol Vis Sci

Publication Year: 2015

Authors: Lyndsay L Leach, David E Buchholz, Vignesh P Nadar, Stefan E Lowenstein, Dennis O Clegg

PubMed link: 25604686

Funding Grants: Synthetic Matrices for Stem Cell Growth and Differentiation, Stem cell based treatment strategy for Age-related Macular Degeneration (AMD), Training Program in Stem Cell Biology and Engineering, Curriculum Development and Implementation of Stem Cell Technology and Laboratory Management Emphasis in an Established MS Biotechnology and Bioinformatics Program at California State University Channel Islands and Co-development of a GE Course on Stem Cel, The UCSB Laboratory for Stem Cell Biology and Engineering, UCSB Stem Cell Biology Training Program, Stem cell based treatment strategy for Age-related Macular Degeneration (AMD), Phase 1 Safety Assessment of CPCB-RPE1, hESC-derived RPE Cell Coated Parylene Membrane Implants, in Patients with Advanced Dry Age Related Macular Degeneration

Public Summary:

The purpose of this study was to better understand the role canonical/beta-catenin Wnt signaling plays in the differentiation of human embryonic stem cells (hESCs) into retinal pigmented epithelium (RPE), with the goal of improving methods for derivation. **METHODS:** Fluorescent reporters were generated to monitor RPE differentiating from hESCs by using a previously described 14-day derivation protocol. Reporters were used to test the effects of the canonical/beta-catenin Wnt pathway agonist CHIR99021 on differentiating RPE. Cells derived from differentiation studies were characterized by lineage-specific transcription factor expression, morphology, pigmentation, and function. The RPE derivation efficiency was determined from percentage positive PMEL17 expression. **RESULTS:** Fluorescent reporters mimicked expression of endogenous genes during 14-day differentiation to RPE. Analysis of Wnt pathway gene expression showed that the pathway components are expressed in differentiating RPE cells. Addition of CHIR99021 improved RPE derivation based on morphology, expression of RPE-specific lineage markers, and genes involved in melanogenesis. Additionally, expression of the neural retina marker CHX10 was suppressed during differentiation with CHIR99021. Addition of soluble WNT3A, but not WNT5A, had the same result. The CHIR99021-modified protocol yielded cell populations that were 97.77% +/- 0.1% positive for the RPE marker PMEL17 at day 14. After cells were expanded to passage 3, they were shown to express RPE markers, carry out phagocytosis of rod outer segments, and secrete pigment epithelium-derived factor apically and vascular endothelial growth factor basally. **CONCLUSIONS:** Our findings demonstrated the importance of canonical/beta-catenin Wnt signaling in RPE differentiation and showed that manipulating the pathway significantly improves RPE derivation from hESC.

Scientific Abstract:

PURPOSE: The purpose of this study was to better understand the role canonical/beta-catenin Wnt signaling plays in the differentiation of human embryonic stem cells (hESCs) into retinal pigmented epithelium (RPE), with the goal of improving methods for derivation. **METHODS:** Fluorescent reporters were generated to monitor RPE differentiating from hESCs by using a previously described 14-day derivation protocol. Reporters were used to test the effects of the canonical/beta-catenin Wnt pathway agonist CHIR99021 on differentiating RPE. Cells derived from differentiation studies were characterized by lineage-specific transcription factor expression, morphology, pigmentation, and function. The RPE derivation efficiency was determined from percentage positive PMEL17 expression. **RESULTS:** Fluorescent reporters mimicked expression of endogenous genes during 14-day differentiation to RPE. Analysis of Wnt pathway gene expression showed that the pathway components are expressed in differentiating RPE cells. Addition of CHIR99021 improved RPE derivation based on morphology, expression of RPE-specific lineage markers, and genes involved in melanogenesis. Additionally, expression of the neural retina marker CHX10 was suppressed during differentiation with CHIR99021. Addition of soluble WNT3A, but not WNT5A, had the same result. The CHIR99021-modified protocol yielded cell populations that were 97.77% +/- 0.1% positive for the RPE marker PMEL17 at day 14. After cells were expanded to passage 3, they were shown to express RPE markers, carry out phagocytosis of rod outer segments, and secrete pigment epithelium-derived factor apically and vascular endothelial growth factor

basally. CONCLUSIONS: Our findings demonstrated the importance of canonical/beta-catenin Wnt signaling in RPE differentiation and showed that manipulating the pathway significantly improves RPE derivation from hESC.

Source URL: <https://www.cirm.ca.gov/about-cirm/publications/canonicalbeta-catenin-wnt-pathway-activation-improves-retinal-pigmented>