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# Lithium increases p63 levels in cultured human limbal epithelial stem cells.

[Agate Noer](#); [Erik O Johnsen](#); [Charlotte L Ness](#); [Aboulghassem Shahdadfar](#); [Bjorn Nicolaissen](#); [Morten Carstens Moe](#)

+ Author Affiliations & Notes

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## Abstract

**Purpose:** Lithium, a known Wnt signaling inducing agent, is added to human LESC cultures ex vivo to reveal whether it enhances the expression of  $\Delta Np63\alpha$  (limbal stem cell marker) and increase stemness in the LESC cultures.

**Methods:** Small limbal biopsies (1x1-2mm) were dissected from donor corneas and cultured ex vivo in a standard medium at 37°C and 5% CO<sub>2</sub> under different concentrations of lithium (LiCl<sub>2</sub>). The cells were cultured for 3-6 weeks and medium was changed every 2-3 days. Immunocytochemistry (ICC), qRT-PCR and Chromatin immunoprecipitation (ChIP) were used to identify the increased number of p63-expressing cells, enhanced  $\Delta Np63\alpha$  expression and epigenetic changes at p63 promoter, respectively.

**Results:** The epithelial cells migrated from the limbal biopsy to form an epithelial sheet. Lithium enhanced the expression of  $\Delta Np63\alpha$ , compared to controls, and this was confirmed by qRT-PCR and ICC. Further studies mapping epigenetic alterations of the lithium treatment are currently conducted.

**Conclusions:** Lithium enhances upregulation of  $\Delta Np63\alpha$  in human LESC cultures ex vivo, and this could be clinically important by increasing the long term success of limbal stem cell therapy by providing cultures and grafts with enhanced stemness and long term proliferative capacity.

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