The Expression of NANO2 and SOX2 During Differentiation Between Human Embryonic Stem Cells and Lung Progenitor Cells

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Introduction

Human embryonic stem cells (hESCs) offer great research and therapeutic potential across multiple fields due to their ability to differentiate into any cell type in the human body. With this unique characteristic, hESCs hold enormous promise in treating diseases such as Parkinson’s, cystic fibrosis, and spinal cord injuries.

For a hESC to maintain its pluripotency and self-renewing state, pluripotent transcription factors such as NANO2 and SOX2 are highly expressed. However, the underlying temporal dynamics of NANO2 and SOX2 during lung progenitor cell (LPC) differentiation still remains poorly understood.

Objective

To address these challenges, the pluripotent transcription factors (TFs) NANO2 and SOX2 were fluorescently tagged to track the gene expression along the endodermal lineage. The expression was then quantified using live-cell imaging under confocal microscopy and analyzed using the single-cell segmentation algorithm Cellpose.

Methods

We used CRISPR/Cas9-mediated gene editing to fluorescently tag the pluripotency genes NANO2 and SOX2, with the mVenus and mTurquoise2 fluorophores, in the H9 hESC line (refer to as H9-NS).

The H9-NS cell line was then differentiated along the endodermal lineage towards lung progenitor cells and imaged using live-cell confocal microscopy.

Results

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A. Endodermal Lineage Differentiation of H9-NS: NANO2 and SOX2 Dynamics During Lung Progenitor Cell Differentiation

B. Single-Cell Segmentation of H9-NS Reporter Cell Line using Cellpose segmentation algorithm. The averaged fluorescent nuclear intensity of NANO2-mVenus and SOX2-mTurquoise2 within individual cells was quantified using Cellpose and visualized in the Napari Image Viewer.

C. Population Fluorescent Level of SOX2 and NANO2 during Lung Progenitor Cell Differentiation

Figure 1. (A) Quantification of NANO2 and SOX2 expression during endodermal differentiation, Live-cell images of NANO2-mVenus, SOX2-mTurquoise2, and merged expression during lung cell differentiation at 0, 12, 24, 36, 48, and 60 hours after differentiation treatment. (B) Single-Cell Segmentation of H9-NS Reporter Cell Line using Cellpose segmentation algorithm. The averaged fluorescent nuclear intensity of NANO2-mVenus and SOX2-mTurquoise2 within individual cells was quantified using Cellpose and visualized in the Napari Image Viewer. (C) The mean nuclear fluorescent intensity of NANO2 and SOX2 during lung progenitor cell differentiation.

Conclusion

It was observed that NANO2 and SOX2 decreased in expression, with an initial loss of SOX2 followed by NANO2, as the H9-NS line differentiated along the lung progenitor cell lineage. Furthermore, NANO2 fluctuates as it decreases in expression, which is observed to vary between individual cells. This gives evidence that this specific lung progenitor cell differentiation protocol creates heterogeneity within the cell population.

Future Directions

To further expand on this experiment, we can utilize immunofluorescently staining with known gene markers for endodermal and lung progenitor cells to access the efficiency and heterogeneity within the population at different stages during the differentiation.

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