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

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Guang Ken Lin, Sonja Mihailovic, Adriana Beltran, Samuel Wolff, Kasia Kedziora, Jeremy Purvis,
Human Pluripotent Stem Cell Core, Department of Genetics, University of North Carolina-Chapel Hill

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.

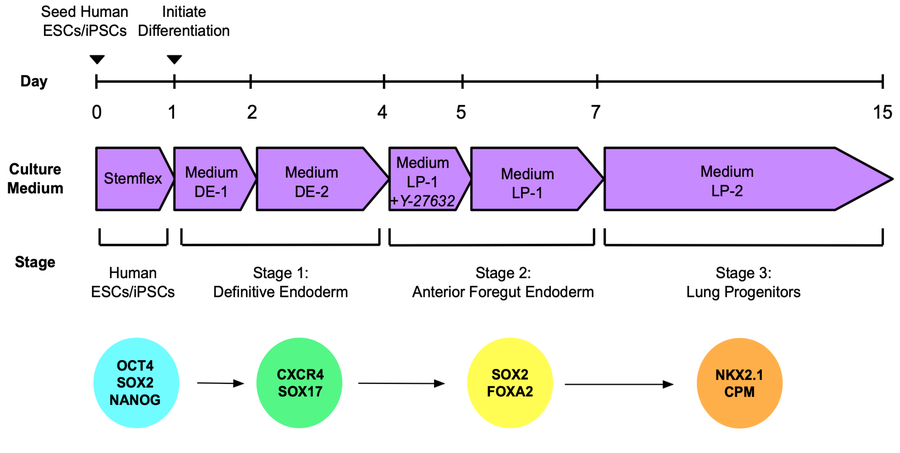
For a hESC to maintain its pluripotency and self-renewing state, pluripotent transcription factors such as *NANOG* and *SOX2* are highly expressed. However, the underlying temporal dynamics of *NANOG* and *SOX2* during lung progenitor cell (LPC) differentiation still remains poorly understood.

Objective

To address these challenges, the pluripotent transcription factors (TFs) *NANOG* 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 *NANOG* 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

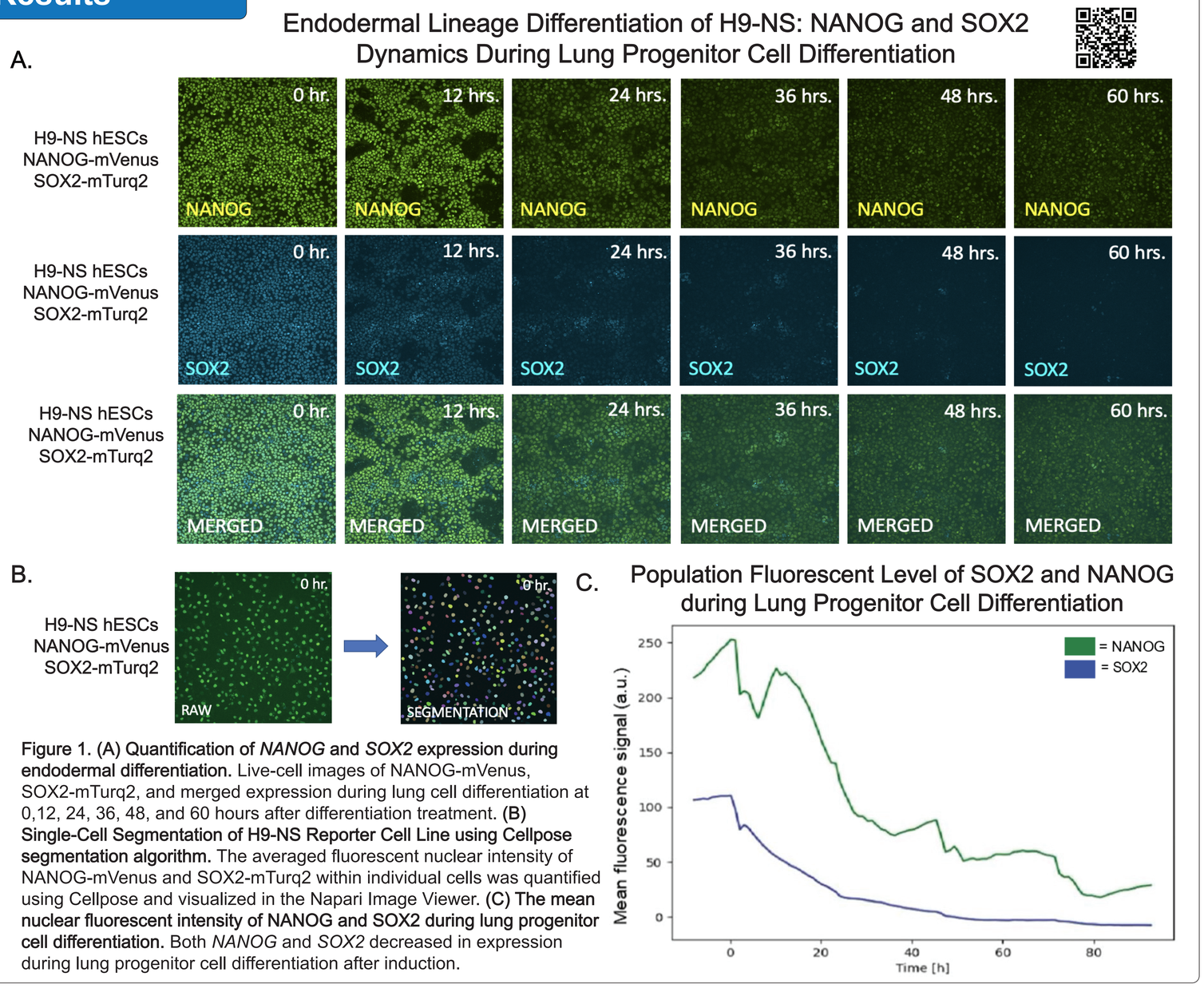


Figure 1. (A) Quantification of *NANOG* and *SOX2* expression during endodermal differentiation. Live-cell images of NANOG-mVenus, SOX2-mTurq2, and merged expression during lung cell differentiation at 0,12, 24, 36, 48, and 60 hours after differentiation treatment. **(B) Single-Cell Segmentation of H9-NS Reporter Cell Line using Cellpose segmentation algorithm.** The averaged fluorescent nuclear intensity of NANOG-mVenus and SOX2-mTurq2 within individual cells was quantified using Cellpose and visualized in the Napari Image Viewer. **(C) The mean nuclear fluorescent intensity of NANOG and SOX2 during lung progenitor cell differentiation.** Both *NANOG* and *SOX2* decreased in expression during lung progenitor cell differentiation after induction.

Conclusion

It was observed that NANOG and SOX2 decreased in expression, with an initial loss of SOX2 followed by NANOG, as the H9-NS line differentiated along the lung progenitor cell lineage. Furthermore, NANOG 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.

Funding Source

University of North Carolina-Chapel Hill, Office of Undergraduate Research, Summer Undergraduate Research Fellowship (SURF), Department of Genetics, Purvis Lab