Effect of Cell Density on Wnt-induced Nodal Signaling in Human Embryonic Stem Cells

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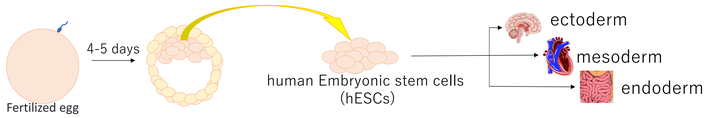
INTRODUCTION

hESCs as an *in vitro* model of early embryonic development

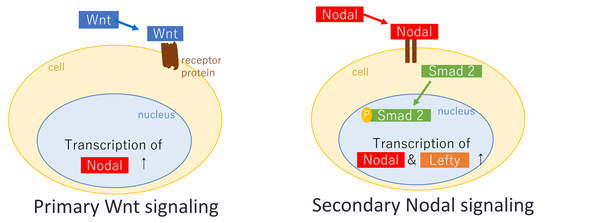


Human embryonic stem cells (hESCs) is a good model to study development

- hESCs are easier to get images and analyze compared to 3D complex embryos
- hESCs are specific to human development

We focused on Wnt and Nodal signaling

Wnt and **Nodal signaling** are important pathways in human embryo that differentiate cells towards mesendoderm fate. However, how they determine cell fates is not completely understood.



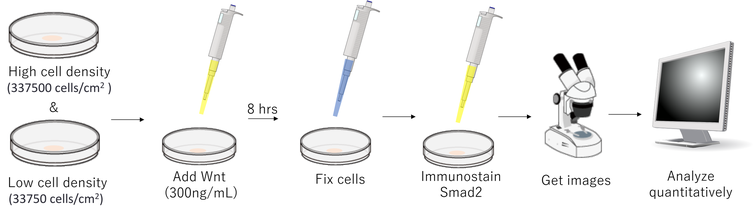
Primary Wnt signaling | Secondary Nodal signaling

How does CELL DENSITY affect Nodal signaling ?

We studied the role of **cell density** on Nodal signaling induced by Wnt signaling. We tested the hypothesis that higher cell density would activate secondary Nodal signaling. This could be because of higher local concentration of Nodal proteins around cells. We used nuclear Smad2 levels as a measure of active Nodal signaling.

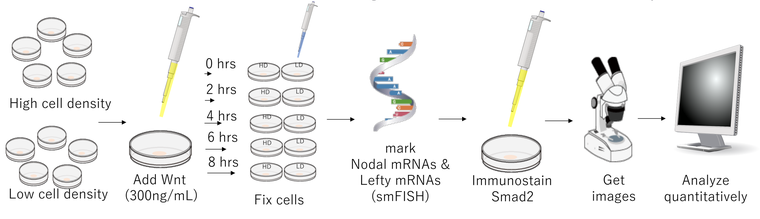
METHODS

TEST1: Does cell density affect the Nodal signaling?



TEST2: What is the timescale of the activation of Nodal signaling?

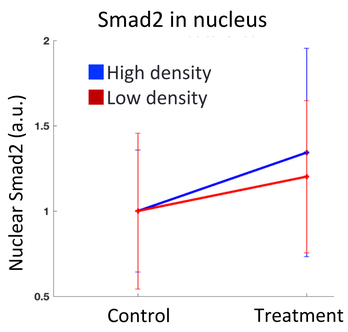
*smFISH: single molecule fluorescent in situ hybridization

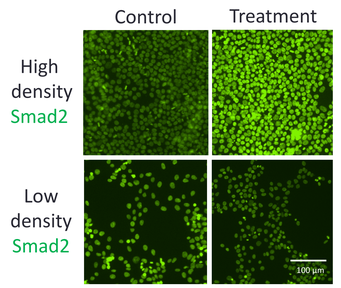


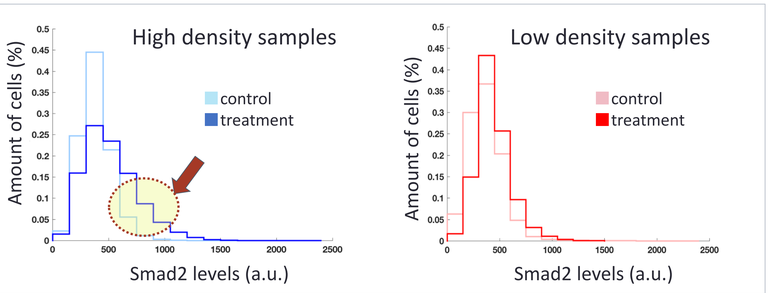
(Some pictures are from http://www.kenq.net/ill/index.html)

RESULTS

TEST1: nuclear Smad2 increased in the high density sample

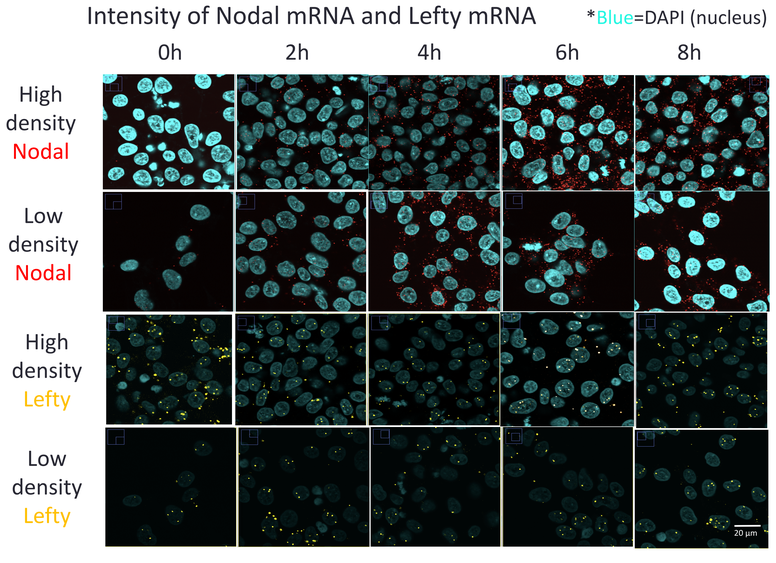


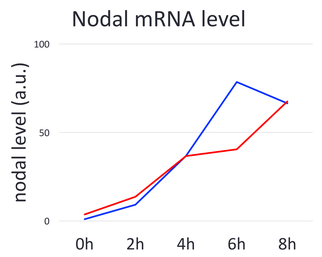


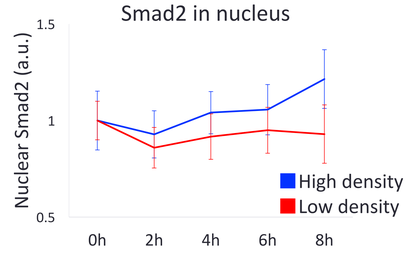


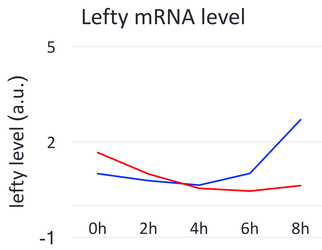
- High density: Intensity of Smad2 in nucleus increased
- Low density: Intensity of Smad2 in nucleus remained the same

TEST2: Lefty mRNA increased only in high density samples Nodal mRNA increased in both samples









Nodal mRNA level
- High density: increased at 0-6 hrs
- Low density: increased at 0-8 hrs

Lefty mRNA level
- High density: increased at 6-8 hrs
- Low density: showed no increase

Smad2 in nucleus
- High density: increased at 2-8 hrs
- Low density: showed no increase

DISCUSSION

- Smad2 in the nucleus increased in high density samples and remained the same in low density samples. Also, Lefty mRNA increased only in the high density samples. This implies that secondary Nodal signaling was not activated in low density samples.
- Although Nodal is a target gene of both primary Wnt signaling and secondary Nodal signaling, Lefty is a target gene only of the secondary Nodal signaling. Therefore, we could say second-signal-induced transcription occurred at 6-8 hrs after the treatment.
- Hence, the higher Nodal transcription between 4-6 hrs in the high density samples is due to high Wnt signaling response. This implies that cells in the high density samples respond better to primary Wnt signaling by producing more Nodal proteins, which then lead to higher Nodal signaling.
- Thus, we conclude that **cell density affects Wnt-induced Nodal signaling.**

NEXT STEPS

Our results suggest that cells in higher cell density sample respond better to Wnt signaling. We can test this hypothesis in two ways:

1) In the same experiment, stain for β-Catenin at different time points.
2) Perform the same experiment in the presence of an inhibitor of Nodal signaling.

REFERENCES

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