Mechanistic Insights into Early Endoderm Differentiation of Human Embryonic Stem Cells using Systems Analysis of Signaling Interactions

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Human embryonic stem cells (hESCs) are an attractive raw material for regenerative medicine due to their potential to deliver a variety of clinically important mature lineages. Several experimental studies have identified the signaling players that govern endoderm lineage specification of hESCs, however the precise mechanisms by which these molecules work together to orchestrate the dynamics of this process are not clearly known. Using a combination of mathematical modeling and model informed experiments, we evaluated the systems level interactions in the TGF-β/SMAD pathway and the role of crosstalks with the self-renewal pathway (PI3K/AKT) in controlling signal propagation and variability during differentiation.

Keywords **— SMAD-AKT crosstalks, Endoderm differentiation, Parametric ensembles, Global sensitivity analysis, Dynamic Bayesian Networks.**

I. Background

THE process of endoderm differentiation in hESCs is initiated by elevating the levels of signaling molecules called SMADs by adding Activin A to the growth medium. However, this alone is not sufficient, the context of the survival pathway PI3K/AKT is extremely important in determining the efficiency of differentiation. Particularly, the signaling activity of this pathway has to be inhibited to get high endoderm differentiation. But this comes at a cost of high cell death. In this work, we evaluated the nature of signaling interactions that govern the balance of signaling interactions during the entire differentiation process.

II. Materials and Methods

A. Experimental setup and analysis

H1 hESCs were maintained on matrigel-coated plates in mTeSR1 and endoderm differentiation was performed using 100 ng/ml Activin A ± modulation of PI3K/AKT pathway using Wortmannin. The phosphorylation dynamics was measured using MagPix multiplex technology. The initial selection of key molecules was based on the study by Singh *et al.* [1]. Nucleo-cytoplasmic shuttling rates were measured using Fluorescence Recovery After Photobleaching (FRAP).

B. Quantitative analysis

We first employed Dynamic Bayesian Network Analysis (DBN) to identify possible interactions from the signaling time series [2,3]. Detailed mechanistic Ordinary Differential Equation (ODE) model for the TGF-β/SMAD2,3 pathway with crosstalk interactions of PI3K/AKT was then developed for a systems level analysis. The model was calibrated using Affine Parallel Tempering based MCMC to identify parametric ensembles and sensitive reactions were identified by meta-model based Global Sensitivity Analysis (GSA) [4].

III. Results and Discussion

Application of DBN on the experimental signaling dynamics showed that the molecules p-SMAD2, SMAD4 and p-SMAD3 are influenced by p-AKT in the early phases of the signaling dynamics. This crosstalk is removed under PI3K inhibition, in spite of recovery of p-AKT levels back to the basal levels. Further, the receptor RII levels influenced the downstream molecules during the entire phase of the signaling dynamics. hESCs further showed divergence in the dynamics of regulatory SMADs. When evaluating this divergence using a mechanistic model, the parametric ensembles of p-SMAD2 and p-SMAD3 showed that there are differences in negative regulation by the negative feedback molecule SMAD7. Further, time to peak response of SMAD2 and SMAD3 was sensitive to the receptor levels and negative feedback. This affects the variability in the availability of SMAD molecules in the nucleus and the nuclear shuttling rates, ultimately controlling the variability seen during differentiation.

IV. Conclusion

Our analysis showed that modulation of crosstalk interactions in combination with inherent variability of specific signaling nodes affects the differentiation response of hESCs. This approach provides a new avenue for rational design and optimization of differentiation media of hESCs.

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References

[1] Singh AM, *et al.* (2012) *Cell Stem Cell* **10**, pp. 312-326
[2] Azhar N, *et al.* (2013) *PLoS ONE* 8: e78202
[3] Mathew S, *et al.* (2015) *MDPI Processes*, under review
[4] Mathew S, *et al.* (2014) *Bioinformatics*, **30**(16), pp. 2334-42