Integrative Proteomic and MicroRNA Approaches Reveal a Novel Post-Transcriptional Motif Regulating Human Definitive Endoderm Differentiation

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***Short Abstract* — Systematic investigation of the developmental stage from human embryonic stem cells (hESCs) to definitive endoderm (DE) may shed light on the underlying mechanisms of human liver development. Here, using two-dimensional difference gel electrophoresis in conjunction with mass spectrometry, we identified two significantly inversely altered splicing-related gene products during the differentiation process, heteronuclear ribonucleoprotein A1 (hnRNP A1) and KH-type splicing regulatory protein (KHSRP). Combined bioinformatics and microRNA-Array data analysis suggests hnRNPA1 and KHSRP antagonizing each other through miR-375 and miR-135a respectively. Further mathematical modeling analysis demonstrated that this motif could generate switch-like responses to the differentiation signal, which can serve as a noise filter to control hESCs self-renewal and differentiation. Simulations predicted that elevated hnRNP A1 or miRNA-375 expression lead to rapid and efficient differentiation of hESCs into DE was further experimentally validated. Taken together, we revealed a potential mechanism which functions in post-transcriptional level to regulate stem cell differentiation.**

***Keywords* — Stem cell differentiation, miRNA, post-transcriptional regulation.**

I. INTRODUCTION

Human embryonic stem cells (hESCs) can self-renew and differentiate into any cell type found in the three embryonic germ layers, making them an attractive source of cells for use in regenerative medicine. The ability to efficiently generate definitive endoderm (DE), the precursor cell type to the liver, pancreas, lungs, thyroid, and intestines, is of great clinical importance. However, differentiation of hESC towards DE is a complicated process and the underlying mechanism remain elusive.

Directing embryonic stem cell differentiation towards definitive endoderm has been achieved by manipulating the Nodal and Wnt signaling pathways. Activin A, which activates the Nodal pathway, directs DE formation from mesendoderm precursors in mouse and human ESCs. In human ESCs, synergistic activation of Nodal and Wnt–β-catenin signaling promotes more efficient DE generation.

While most previous studies in this area have focused on identifying gene expression and signaling pathways, we chose to investigate the key proteins associated with the differentiation process. Here, we report results of a comparative proteomic analysis on DE derived from hESCs in feeder layer-free conditions, using two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry (MS), followed by bioinformatics and mathematical modeling analysis on the function of identified regulatory motif. The identified post-transcriptional level motif may shed light on the underlying mechanism of hESC fate decision.

II. CONCLUSION

Combined experimental and modelling tools, we identify a possible post-transcriptional motif regulating DE differentiation. The newly identified motif could generate switch behavior corresponding to the differentiation. Perturbing the system with hnRNPA1 and miR375 could promote differentiation. The post-transcriptional level switch may serve as a noise filter for ESC differentiation regulation which may prevent inadvertent differentiation by random exogenous signal fluctuations.

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