Program/Abstract #369
Role of FoxD3 in maintenance of pluripotency and early lineage segregation in human embryonic stem cells
Brigitte Arduini, Ali H. Brivanlou
The Rockefeller University, Lab of Molecular Embryology, New York, NY, USA

Among the earliest fate decisions in mammalian development are those between extraembryonic tissues, including primitive ectoderm and endoderm, and the epiblast, which gives rise to the embryo proper. Subsequently, epiblast cells generate definitive endoderm, mesoderm, and ectoderm. The transcription factor FoxD3 has been intimately linked to extraembryonic ectoderm (trophectoderm), definitive endoderm and mesoderm development, cell fate diversification in the neural crest, and maintenance of mouse epiblast and embryonic stem cells. Yet virtually nothing is known about its role in human development. Using stable transgenesis in human embryonic stem cells (hESCs), we have undertaken conditional gain- and loss-of-function analyses of the role of FoxD3. Knockdown of FoxD3 in conditions that otherwise maintain pluripotency leads to down-regulation of pluripotency genes and morphological changes in hESCs. These changes are accompanied by increases in genes expressed by definitive endoderm and mesoderm, as well as decreases in colony growth rate. Further, loss of FoxD3 function abrogates BMP4-induced differentiation of trophectoderm from hESCs. Similarly, overexpression of FoxD3 causes significant reduction in oct4 and nanog expressions. These cells upregulate snail2 expression, a hallmark of epithelial-to-mesenchymal transition, undergo dramatic morphological changes, and are loosely packed compared to the tight conformation of undifferentiated hESC colonies. Together, these data suggest that an optimal level of FoxD3 expression is essential for maintenance of pluripotent hESCs, and that tipping this balance in either direction leads to rapid differentiation toward distinct early embryonic lineages.

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Program/Abstract #370
ZNF 281 decides the early differentiation fate of human mesenchymal stem cells
Kwang-won Seo[a], Seon-Kyung Lee[b], Dilli Ram Bhandari[b], Sang-Bum Park[b], Kyung-Hwan Roh[b], Se-Ran Yang[b], Kyung-Sun Kang[b]
[a]*Seoul National University College of Vet. Med., Adult Stem Cell Research Lab, Seoul, Republic of Korea*
[b]*Seoul National University, Seoul, Republic of Korea*

ZNF281 is one of the core transcription factors in embryonic stem cells (ESCs), which has activation and repression roles in the transcription of ESCs' genes. A known target molecule of Zfp281 (mouse homologue of ZNF281) is Nanog. However, NANOG does not express in almost human mesenchymal stem cells (hMSCs). Here, we showed the roles of ZNF281 with the gain and loss of function study. The knockdown of ZNF281 resulted in spontaneous osteo-chondrogenic differentiation and reduced the proliferation of hMSCs, which were confirmed by in vivo and in vitro experiments using cell morphology and molecular markers. When ZNF281 knocked-down hMSCs were subcutaneously implanted with β-TCP in mice, most of the cells were converted into osteoblasts within four weeks. The over-expression of ZNF281 in hMSCs resulted in accelerated proliferation. The expression pattern of ZNF281 was well correlated with the expression of β-CATENIN during differentiation and gain/loss of function study in hMSCs. The binding of ZNF281 to the promoter region of β-CATENIN was confirmed using Chromatin Immuno-Precipitation (ChIP) assay. In conclusion, we propose that ZNF281 is essential to the maintaining of stem cells via transcriptional regulation of genes including β-CATENIN.

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Program/Abstract #371
Polycomb a potential barrier to de-differentiation in somatic plant tissue
Vlad Nicolaescu, Jocelyn Malamy
University of Chicago, Chicago, IL, USA

Terminally differentiated cells lack the ability to differentiate into other cell types, even when given the appropriate differentiation signals. This suggests that a barrier exists in terminally differentiated cells to prevent them from losing their identity, or de-differenting. Understanding the mechanism underlying this barrier will shed light on the process of differentiation during normal development, and will contribute to attempts to de-differentiate somatic cells for the creation of therapeutic stem cells. Plants provide a compelling system to study de-differentiation, as certain plant cells readily de-differentiate in response to plant hormones to create masses of unorganized cells known as "callus". We used the Arabidopsis root to study the barriers to de-differentiation, as only one cell type in the root dedifferentiates to form callus in response to exogenous hormones. Our approach was to look for genes whose mutation leads to "ectopic" callus formation from the other cell types, indicating a release of the de-differentiation barrier. We hypothesized that the Polycomb group proteins (PcG) may play a role in preventing cell dedifferentiation as certain PcG mutants in Arabidopsis were previously reported to show hallmarks of spontaneous cell de-differentiation. Excitingly, we found that mutation of the polycomb protein LHP1 leads to ectopic callus formation. In addition, defects in LHP1 and other PcG genes increased callus formation in its normal location. These results indicate that, in Arabidopsis, PcG is an essential component of the barrier to cell de-differentiation.

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The role of Smad4-dependent signaling in mouse trophoblast stem cells
Jiami Guo, Padhmavathy Yuvaraj
Kent State University, Kent, OH, USA

Mouse trophoblast stem (TS) cells established from the outgrowth of the polar trophectoderm of a blastocyst or the extraembryonic ectoderm of a post-implantation embryo can contribute to all trophoblast lineage derivatives in vivo, providing a powerful in vitro system for the study of trophoblast stem cell self-renewal and differentiation. Although it is known that Transforming Growth Factor (TGF)-beta/Nodal-related signaling together with FGF4 signaling is critical for TS self-renewal, the function of Smad4, the central mediator of TGF-beta signaling pathway, in TS cell is not well understood. To investigate the role of Smad4-dependent signaling in trophoblast lineage development, we derived smad4 null TS cells