# 23rd Stem Cell Club Meeting

## ESC pluripotency and differentiation

*(Organised by the Stem Cells Research Singapore Website Committee http://www.stemcell.edu.sg)*

Date: May, 16th 2007 (Wednesday)
Time: 5:30 pm
Venue: Matrix, Exploration Theatrette, Level 2M

Host: Justine Burley, NUS

| Time | Title | Speakers |
|---|---|---|
| *5:30-6:10* | *Zic3 regulates embryonic stem cell pluripotency by preventing endodermal lineage specification* | **Lim Shusan Linda** *GIS* |
| *6:10-6:50* | *Deriving functional insulin-producing cell lines from mouse embryonic progenitors including ESCs* | **Lim Sai Kiang** *IMB* |
| **7:00-** | **Wine and Cheese (at Invitrogen facilities, 4th floor, Chromos)** | |

# Zic3 regulates embryonic stem cell pluripotency by preventing endodermal lineage specification

Linda Shushan Lim, GIS

Dissecting the transcriptional networks operative in embryonic stem (ES) cells will provide insights to the properties of stem cells that allow unlimited growth and differentiation. How do we begin to tease apart the intricate networks that extend beyond Oct4, Nanog and Sox2, transcription factors that are known to regulate pluripotency? The first clues can be found among genes that are differentially expressed between the pluripotent and early differentiation phases, where changes in gene expression imply a potential role for these genes. Additional insights into this regulatory network was provided by genome-wide mapping of Oct4, Nanog and Sox2 binding sites in mouse and human ES cells by chromatin immunoprecipitation (ChIP). Together these data provide an opportunity to identify additional players and establish direct links among various components of the regulatory network.

Our analysis of ES cell genomic data has identified Zic3, a zinc finger-containing transcription factor, as a component of the regulatory network and its relationship with other pluripotency factors. The regulatory region of *Zic3* is bound by Oct4, Nanog and Sox2 in both mouse and human ES cells. Zic3 is highly expressed in ES cells and repressed upon differentiation. Questions arising from these data are: How do the key regulatory factors interact with Zic3, and what results from this interaction? What role does Zic3 play in the embryonic stem cell? How does the absence of Zic3 affect cells during the early differentiation stages?

The above questions have been addressed using a loss-of-function approach for Zic3 in mouse ES cells. Knock-down of Zic3 expression by RNA interference induced differentiation towards endoderm lineage. We propose a role for Zic3 in the maintenance of pluripotency by functioning as a gatekeeper blocking the differentiation of ES cells into endoderm. Notably, the expression of Nanog, a key pluripotency regulator and repressor of extraembryonic endoderm specification in ES cells, was significantly reduced in Zic3 knockdown cells. This suggests that Zic3 may prevent endodermal specification through Nanog-regulated pathways. Further work seeks to clarify the molecular interactions of Zic3 that mediate its function as a regulator of pluripotency. In this manner, our approach to the elucidation of molecular signatures of early embryonic stem cells will contribute to validation and extension of the ES cell transcriptional network beyond Oct4, Nanog and Sox2.

## Deriving functional insulin-producing cell lines from mouse embryonic progenitors including ESCs

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Generating large numbers of homogenous insulin-producing cells from ESCs has been difficult. We circumvented this problem by generating insulin-producing cell lines. From an empirical derivation of insulin producing cell lines, MEPI-1 to 14 from mouse embryos, we progressed systematically and incrementally to the derivation of RoSH2.K from embryo-derived RoSH2 cell line, and then to gleevec-insensitive E-RoSHK lines from mouse ESC-derived $CD9^{hi}$ $SSEA\text{-}4^{-}$, and gleevec-sensitive E-RoSH cell lines. Insulin content was ~8 µg µg/$10^6$ MEPI-1 cells and 0.3-3.4 µg /$10^6$ RoSH2.K or E-RoSHK cells. All the lines have similar gene expression profiles, expressed β cell-specific genes including insulin-1 and insulin-2, stored and secrete equimolar ratio of insulin and C-peptide, and secrete insulin through a glucose-sensitive β cell-like secretory machinery. Transplanted MEPI-1, RoSH2.K or E-RoSHK cells reversed hyperglycemia in streptozotocin-treated SCID mice but did not induce teratoma formation.