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学位論文題目	Regulation of Transcriptional Heterogeneity in Pluripotent Stem Cells (多能性幹細胞における転写不均一性の制御)

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論文の要旨
Abstract of thesis

(Background/Purpose)

Two pluripotent stem cells (PSCs): embryonic stem cells (ESCs) and epiblast stem cells (EpiSCs) represent naïve and primed pluripotent states which exhibit significant differences. PSCs possess the capacity to self-renew and differentiate into various type of cells. Heterogeneity is one of the underlying mechanisms which might keep PSCs to have the capacity to self-renew under various environments. However, how heterogeneity evolves as a molecular basis for PSC self-renewal and undifferentiated remains unclear. The author would like to investigate as following:

- to examine how the heterogeneity of ESCs is regulated focusing on *Rex-1* gene expression which is a well-known pluripotent gene expressed in a heterogeneous pattern

- to investigate whether EpiCSc have different subpopulations that vary in differentiation ability focusing on *Fgf5* gene expression

(Material and methods)

To investigate the mechanisms of regulation of ESCs heterogeneity, the author used a double knock-in cell line, OCRG9 ES cells, where fluorescent genes were inserted into *Oct3/4* and *Rex1* genes, respectively. Then the author examined gene expression between Rex-1-positive and –negative populations, and found significant differences in the expression of glycolysis- and antioxidant-related genes. Therefore, the author used pharmacological and genetic approaches to explore the effects of glycolysis and ROS signaling pathway on the regulation of ESCs heterogeneity.

To examine the heterogeneity in EpiSCs, the author generated *Fgf5* transgenic EpiSCs with *Venus* gene and examined heterogeneous expression of FgF5 at E6.5 and E7.5 embryos.

(Results)

1) The author found that *Rex-1*-negative population show increased expression associated with glycolysis pathway, such as *Glut1, Gapdh, Pgk1, Eno3*, and *Ldha*

2) The author found that suppression of glycolysis pathway using glycolysis inhibitors, 2-DG and 2-FDG affected the temporal interchange of intercoversion of Rex-1 positive and Rex-1-negativie cells

3) The author found that *Hk2* gene-knocked out ESCs showed a reduction of *Rex-1*-negative cells, suggesting that glycolysis pathway is an intrinsic factor regulating ESC heterogeneity

4) The author found that ROS generation downstream of glycolysis inhibition is an essential factor regulating the process of the conversion between *Rex-1*-positive and –negative

5) The author found that the reduction of ROS inhibited the re-establishment of ESC heterogeneity from both *Rex-1*-positive and –negative cells

6) The author found that *Fgf5*-positive EpiSCs showed mesodermal markers while *Fgf5*-negative EpiSCs showed ectodermal markers

審査の要旨
Abstract of assessment result

【批評 Review】

The author showed us that *Rex-1*-negative cells preferentially possessed glycolysis pathway with cell survival activity rather than *Rex-1*-positive cells. Of note, the author demonstrated that glycolysis inhibition abolished the *Rex-1*-positive cell enrichment derived from *Rex-1*-negative cells, indicating the importance of the glycolysis in the heterogeneity of ESC development.

We asked the author about the physiological and scientific importance of metabolic changes from the sate of ESCs to EpiSCs and the author clearly explained the biological importance of the existence of heterogeneity situation during the early development of pluripotent stem cells. We also asked the author the method how to detect the measurement of metabolic changes besides the changes of metabolic gene transcriptions. The author showed us the possibility of the measurement that would be useful to detect.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on January 13th, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.