Results report

１． Title of Research and Development : Genetic and epigenetic hierarchies distinguishing pluripotent and trophoblast stem cells

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４． Results of Research and Development:

In this fiscal year, we finalized the standard protocol for analyzing the transcription factor-mediated transition of embryonic stem (ES) cells to trophoblast stem (TS) cells that is optimal for the multiple types of omics analyses. Then we applied this protocol to prepare the DNA, RNA and chromatin samples at day 0, 3 6 of transition culture for further analyses. Using the chromatin sample, chromatin immuno-precipitation (IP) was performed by the Niwa team for Oct3/4, Sox2, Cdx2, Eomes and Elf5 and the sequencing data was obtained from these IP samples. For DNA methylation analysis, PBAT protocol was optimized for the analysis of the time-course samples in the next fiscal year.

To reveal the functions of epigenetic regulators in ES-TS transition, we developed a new system to achieve a dual control of Cdx2 activity and Cre activity for induction of differentiation and knockout event, respectively. In this system, we use a chimeric ligand binding domain of the mutant progesterone receptor to control the activity of Cdx2 by RU486. The new tool, Cdx2GPRh, is regulable without any interaction to the acivation of MerCreMer by tamoxifen. We introduced this system into the ES cell lines carrying the flox alleles of G9a, Setdb1 and Dnmt3a+Dnmt3b and established the cell lines in which the regulation of the transgene activities was confirmed.

In parallel to this comprehensive approach, the candidate approach using primate cells was performed for generating human TS-like cells. It was well known that the activation of the transcription factor Cdx2 is sufficient to trigger transition of mouse ES cells to TS cells. In contrast, when the impact of CDX2 activation was assessed in human ES cells by Ko team, it was not sufficient to activate the transcriptional profile similar to human placental cell lineage. Instead, several transcription factors were identified for their ability to trigger the transcriptional profile similar to human placental cell lineage after induction of their expression in human ES cells. Now the impact of the combinatorial expression of these transcription factors is accessing for induction of differentiation toward TS-like cells.

In last fiscal year, Tanaka team succeeded to isolate trophectodermal cell lines from the blastocyst-stage embryos of macaque monkey. They performed characterization of these cell lines and found that they showed the gene expression pattern characteristic to trophectoderm lineage by changing the culture condition. Moreover, they possessed the hyper-methylated DNA in the region differentially methylated in mouse TS cells. They also tried to repeat the establishment in the modified culture condition and succeeded to generate three additional trophectodermal cell lines from macaque blastocysts.

The Japanese team members communicated to the Canadian members to establish the standard protocol for ES-TS transition. The assessment of the impact of CDX2 expression in human ES cells and the analysis of the transcriptome data of macaque trophectodermal cell lines were performed in collaboration with the Canadian members.