〔課程-2〕

審 査 の 結 果 の 要 旨

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The dissertation describes a disease modeling for Wiskott Aldrich Syndrome (WAS) using patient-derived induced pluripotent stem cells (iPSCs). Wiskott Aldrich Syndrome is an X-linked disease caused by mutations in the gene encoding the WAS protein (WASp). This disease is characterized by thrombocytopenia, immunodeficiency and eczema. Thrombocytopenia is one of the most important feature of this disease, which is associated with a significant risk of life-threating hemorrhage. Because of limitations in modeling of this rare disorder, precise mechanisms of the platelet abnormality remains to be elucidated. In this study iPSC lines were established using CD34+ cells of peripheral blood from two XLT and one WAS patient by utilizing sendai virus harboring four Yamanaka factors (SOX2, KLF4, OCT3/4 and c-MYC). This disease-specific iPSC lines in combination with our protocol of differentiation of iPSCs to the hematopoietic progenitor cells and fully differentiated megakaryocytes and platelets gave a unique opportunity to address the issue of platelet abnormality in these patients. Some important finding of this study are as follows:

1- Characterization of iPSC lines revealed that iPSC clones retained typical characterization of pluripotent stem cells and they retained gene mutations characteristic to each patient.

2- Absolute numbers of megakaryocytes and platelets -showing double positive CD41 and CD42 cell surface markers- obtained from the same numbers of hematopoietic progenitor cells (CD34+ CD41+) were significantly smaller in both XLT- and WAS-iPSCs comparing to those from healthy-iPSCs. Also it was demonstrated that the number of proplatele-bearing cells obtained from same numbers of progenitor cells is significantly reduced in patient samples comparing to the healthy counterpart.

3- This study demonstrated that megakaryocytes of patients are phenotypically normal in our culture system and have the ability to produce comparable numbers of platelets

comparing to the healthy donor samples suggesting that platelet production ability of megakaryocytes is not affected by WASp deficiency and reduced numbers of platelets which are observed in our culture system is due to reduced number of proplatelet-bearing cells.

4- Colony formation ability of both XLT- and WAS-iPSCs –derived Hematopoietic progenitor cells (HPC) were tested and data revealed that the ability of patient-HPC for differentiation to hematopoietic lineage is affected by WASp deficiency and it varies between patient samples and depends on the distinct type of mutation.

5- This study revealed that WASp is involved in TPO signaling pathway in the hematopoietic progenitor cells differentiation toward megakaryocytes. As a dull phosphorylation pattern for downstream molecules of MPL was seen for WASp null progenitor cells by FACS analysis, this study suggests that WASp expression is important but dispensable for TPO signaling.

6- Lentiviral-mediated gene transfer led to appearance of WASp expression in patient iPSC-derived megakaryocytes. Although the expression level of WASp did not reach the normal level that was seen in control-iPSC-megakaryocyte; it seemed to be sufficient to significantly increase yields of platelets and megakaryocytes comparying to control vector after gene transfer.

This study provides new insight into the mechanism underlying thrombocytopenia in Wiskott Aldrich Syndrome and plays an important role in improvement of treatments for the patients in future.