博士論文（要約）

Establishment of mass production of functional neutrophils from human iPSCs and analysis of myeloid progenitor differentiation

（ヒト iPS 細胞由来機能性好中球の大量産生法の確立、及び骨髄系細胞分化機構の解析）

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Neutrophils play an essential role in innate immune response to bacterial and fungal infections, thus neutropenia caused by chemotherapy or hematopoietic stem cell transplantation readily leads to lethal infections in clinical practices. Despite the development of antibiotics, infections remain as the main cause of death in patients suffering from hematological malignancies. Granulocyte transfusion therapy is a promising therapeutic option besides antibiotics, but its physical burden on donors is the main obstacle. Neutrophils derived from human induced pluripotent stem cells (iPSCs) may become the alternative source, but the method of generating expandable neutrophils sufficient for clinical use has not been established yet.

Here I show that engineered neutrophil-primed progenitors derived from human iPSCs achieve the clinically applicable scale of functional neutrophils, which act promptly against bacterial infection. After differentiating iPSCs into hematopoietic stem and progenitor cells, I overexpressed *c-Myc*, *BMI-1*, and *BCL-XL* in a doxycycline-inducible manner with culture mediums that included TPO, SCF, and Flt3-L followed by gradual replacement with G-CSF. This protocol generated expandable iPSC-derived neutrophil progenitors, and after removing doxycycline, these progenitors differentiated into mature neutrophils in 4 days. I checked adhesion, migration, phagocytosis, and bacterial killing capacity *in vitro* and showed these neutrophils can exert antibacterial activity that is similar to primary human neutrophils. RNA

sequencing data showed that mature neutrophil signature gene set was upregulated after removing doxycycline. Also, LPS stimulation induced the upregulation of inflammatory pathways. As for *in vivo* analysis, I performed *in vivo* imaging of luciferase-expressing neutrophils in mice and showed these neutrophils promptly migrated to the infection site after intravenous injection. Moreover, I used lethal acute peritonitis models to address the significance of neutrophils on survival. Intraperitoneal injection of neutrophils in the acute peritonitis model caused by *S. aureus* improved survival in a dose-dependent manner. This system would provide the straight-forward solution, off-the-shelf transfusion of neutrophils for patients suffering from neutropenia, and the platform for analysis of human neutrophil involved in broad spectrums of physiological and pathological conditions.

Also, analyzing the differentiation mechanism of neutrophils provides new insights into innate immunity. The differentiation of hematopoietic cells is strictly regulated in a hierarchical manner. Common myeloid progenitors (CMPs) differentiate into granulocyte-monocyte progenitors (GMPs) and megakaryocyte-erythrocyte progenitors (MEPs), and GMPs generate neutrophils and monocytes. Recent studies revealed that each progenitor population has significant heterogeneity and some of the subsets have skewed differentiation potential. By analyzing single-cell RNA sequencing data of human and murine CMPs and GMPs, I newly identified CD62L (L-selectin) as a marker to refine the differentiation

potential of CMPs and GMPs. At CMP level, I clarified that CD62L-low CMPs had genuine CMP potential, whereas CD62L-high CMPs were mostly skewed to GMP potential in both mice and humans. At GMP level, I found that CD62L-neg GMPs were the most immature subset in GMPs and part of them remained CMP potential. Also, CD62L-low GMPs were skewed to neutrophil differentiation. These findings provide the more profound understanding of the differentiation mechanism of neutrophils, and elucidate the mechanism of innate immunity.