【Grant-in-Aid for Scientific Research (S)】

Broad Section I

Title of Project： Analysis of stemness, aging and carcinogenesis using hematopoietic stem cell ex vivo amplification system

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Keyword：hematopoietic stem cell, ex vivo expansion, clonal hematopoiesis, CRISPR gRNA genome wide screening, multiomics analysis

【Purpose and Background of the Research】

Hematopoietic stem cells (HSCs) have been well studied for a long time. However, the details of the regulatory mechanisms of differentiation and self-renewal, which are the fundamental principles of stem cell biology, remain unclear due to the paucity of HSCs in the bone marrow. Most recently, we have developed and reported a method of long-term in vitro culture of mouse HSCs, a long-held dream of hematology, to expand more than 900-fold in 4 weeks while maintaining stem cell function (Wilkinson et al. Nature 2019). In this study, using the long-term HSC expansion culture method, we will attempt genome wide CRISPR screening for HSCs and mutation analysis after long-term culture. We will approach the elucidation of the mechanism of differentiation and self-renewal as well as the pathogenic mechanism of age-related hematological malignancies. Furthermore, we aim to establish a culture method that enables ex vivo expansion of human HSCs and achieve the Holy Grail of hematology.

【Research Methods】

In our ex vivo mouse HSC expansion, the purity of functional stem cells gradually decreases with long-term culture. First, FACS and transplantation experiments will be used to identify surface markers that can purify functional HSCs from the expanded cells.

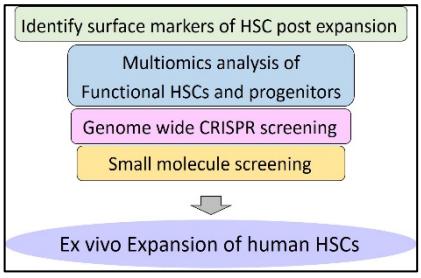

Figure 1 Research strategy

Based on this result, the true stem cell fraction is isolated and a multi-omics analysis that requires a large number of samples is performed. At the same time, comprehensive gene knockout will be performed with the CRISPR / Cas9 genome editing library to identify the signals required to maintain stem cell properties.

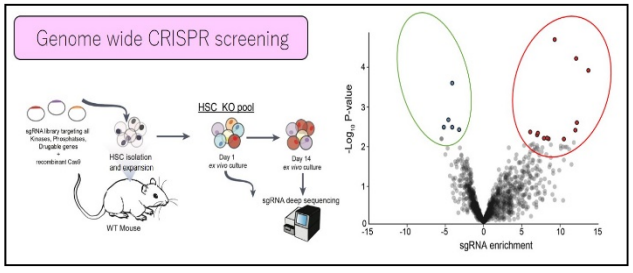

Figure 2 Genome wide CRISPR screening for HSCs

Screening for small molecule compounds, etc. will also be carried out, focusing on the identified signaling pathways. Then, by applying the obtained findings to the culture system of human hematopoietic stem cells, we aim to elucidate the nature of self-renewal of hematopoietic stem cells and the conditions necessary for amplification of human HSCs.

【Expected Research Achievements and Scientific Significance】

HSCs have been clinically applied as an established treatment for hematopoietic malignancies and hereditary blood diseases in the form of bone marrow transplantation for more than 50 years, but it is difficult to find HLA-matched donors. In addition, age-related increase in hematological malignancies is strongly correlated with the accumulation of gene mutations, and there is a high frequency of clonal expansion of HSCs in the bone marrow of the elderly, which is considered to be a pre-leukemic state. However, the mechanism from mutation accumulation to onset is unknown. It is expected that these long-standing problems in hematology will be solved by performing multi-omics analysis enabled by the long-term expansion culture system of mouse HSCs that we developed.

【Publications Relevant to the Project】

1. Wilkinson AC, Igarashi KJ, Nakauchi H. (2020). Haematopoietic stem cell self-renewal in vivo and ex vivo. Nat Rev Genet. 21(9):541-554. "PMID": 32467607.
2. Wilkinson AC, Ishida R, Kikuchi M, Sudo K, Morita M, Crisostomo RV, Yamamoto R, Loh KM, Nakamura Y, Watanabe M, Nakauchi H*, Yamazaki S*. (2019). Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. Nature. 571(7763):117-121. "PMID": 31142833.
3. Yamamoto R, Wilkinson AC, Ooehara J, Lan X, Lai CY, Nakauchi Y, Pritchard JK, Nakauchi H. (2018). Large-Scale Clonal Analysis Resolves Aging of the Mouse Hematopoietic Stem Cell Compartment. Cell Stem Cell. 22(4):600-607 e604. "PMID": 29625072.

【Term of Project】

FY2020-2024

【Budget Allocation】

152,600 Thousand Yen

【Homepage Address and Other Contact Information】

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