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研究課題名（和文）CRISPR/Cas9-mediated genome-editing of long-term hematopoietic stem cells

研究課題名（英文）CRISPR/Cas9-mediated genome-editing of long-term hematopoietic stem cells

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研究成果の概要（和文）：GSKとmTORは長期 HSCのHDR 編集効率を向上させることを確認した。β-グルカンを使用して編集細胞の生着を改善する方法を確立した。我々の結果では、β-グルカンがゲノム編集された長期 HSC を保護し、 HDR 編集された長期 HSCの移植後、効率よく骨髄に生着されることが分かった。

研究成果の学術的意義や社会的意義
Genome-edited HSCs engraftment capability is an important point for successful HSCs transplantation. This work shows the ex vivo treatment of beta-glucan before the genome-editing process has protective effects for genome-edited HSCs to survive in the recipient body after transplantation.

研究成果の概要（英文）：To improve in vitro HDR editing efficiency of expanded/edited long-term HSCs, we tested first, GSK and mTOR inhibitors which are known to support the maintenance and self-renewal of HSCs. Treatment with two inhibitors, HDR-mediated editing improved from ~5% to 10-30 % in vitro; from ~1% to ~10% in vivo 2) Modified single-strand DNA template to conjugate the template to Cas9, and it showed 5-20% in vitro; ~5% in vivo after serial transplantation. Second, we established and optimized the method to improve the edited cell engraftment by using beta-glucan. Our result showed that beta-glucan protects HSCs and improves their engraftment ability. These results showed that ex vivo expanded and HDR-edited cells
are capable to repopulate HDR-edited multilineage cells after transplantation, demonstrating that precise genome editing of expanded long-term HSCs is feasible.

研究分野：Regenerative medicine

キーワード：CRISPR/Cas9　Hematopoietic stem cells　HDR　Genome-editing　beta-glucan　mTOR inhibitor　GSK inhibitor　Engraftment

１．研究開始当初の背景

Hematopoietic stem cells (HSCs) have self-renewal and multipotency ability and can give rise to entire blood cells in a body. Life-long blood regeneration is critically dependent on self-renewing multipotent HSCs. Due to their ability, long-term HSCs (LT-HSCs) become an attractive target for genome-editing therapy to treat inherited blood disorders. CRISPR/Cas9 genome editing is a powerful tool to study genotype-phenotype relationships of genome-editing therapy of HSCs and promising therapeutical option for genetic disease. Ex vivo editing and expansion of mouse HSC is a useful method to study the engraftment capacity of genome-edited HSCs. The main goal of genome-edited HSCs transplantation is to inject HSCs with high self-renewal potential that can reestablish the entire blood system.

Ex vivo genome-editing of HSCs has three main settings to optimize, and expansion of HSCs for the enrichment of target cell population, efficient editing of long-term HSCs, and preparing engraftment-capable cells for transplantation. Recently PVA contained medium showed efficient ex vivo expansion of HSCs, which gives possibilities to test the engraftment ability of expanded/edited HSCs in the recipient body. Proliferative culture conditions have been widely used before and after genome-editing. HSCs gradually lose their repopulation capacity upon prolonged exposure to proliferative conditions which highlights the necessity of proliferative culture conditions that maintain HSC function. Thus, to develop a new effective targeted genome repair for HSCs, we focused to improve ex vivo HDR editing and maintain the engraftment-capable genome-editing HSCs ex vivo.

２．研究の目的

To elucidate the hypothesis, I propose two specific aim:

Aim 1: To develop HDR-editing in long-term HSCs by CRISPR/Cas9.

Aim 2: Improvement strategy of in vitro or in vivo HDR editing efficiency of expanded/edited long-term HSCs. In the proposed study, we developed HDR-edited long-term HSC for autologous transplantation.

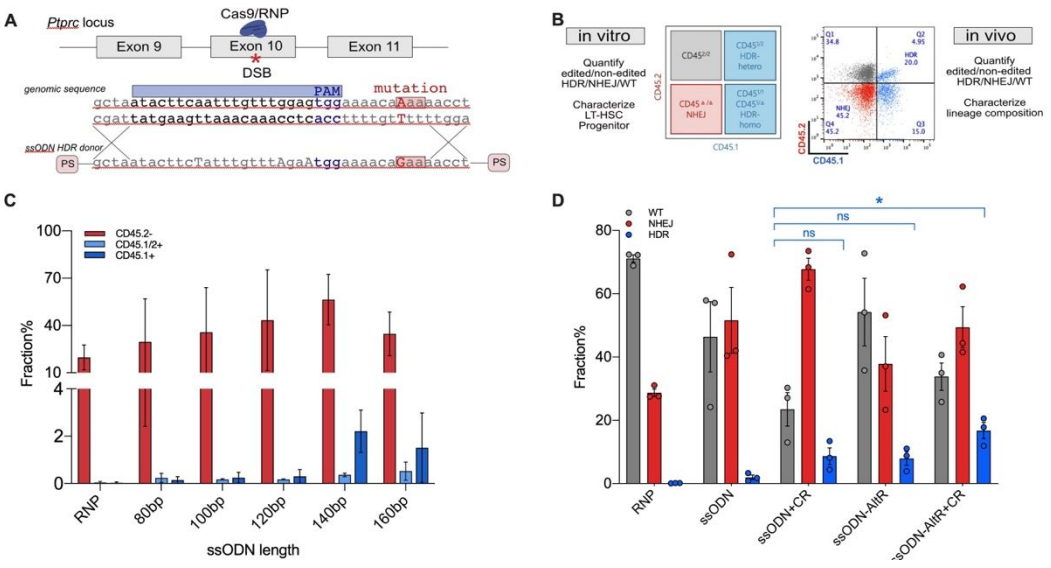

Figure 1. A. A schematic diagram showing the targeted site of gRNAs at the *Ptprc* exon 10 and the ssODN donor template for HDR to cause single amino acid polymorphism in CD45.1 and CD45.2 **B.** A schema to quantify the edited or non-edited cells in vitro and in vivo. Characterize the LT-HSCs or progenitor cells among the edited population in vitro pre-transplantation and characterize lineage composition of edited cells in vivo post-transplantation. **C.** Comparison of the delivery method of 80-160bp of single-stranded DNA repair template with Cas9 ribonucleoprotein (RNP) to LT-HSCs, to make conversion of CD45.2 to CD45.1 in vitro. **D.** CD45.2 to CD45.2 editing with standard ssODN, modified ssODN (ssODN-AltR), or GSK or mTOR treated (CR) groups (n=3 in each).

３．研究の方法

Aim 1: Develop ex vivo expansion of long-term HSCs for genome editing

First, we enriched mouse LT-HSCs by sorting Lineage negative (Lin-), c-Kit+, Sca1+, CD150+, and CD34- cells, and total of 50-500 cells from bone marrow were expanded using PVA-containing media (Dr. Yamazaki) under the fibronectin-coated plate with TPO and SCF cytokines. Long-term HSCs were expanded to 10-50 times in 7 days. Second, we optimized HDR-based genome-editing in expanded LT-HSCs.

Aim 2: Improvement strategy of in vitro or in vivo HDR editing efficiency of expanded/edited long-term HSCs

To improve HDR-mediated editing in LT-HSCs, we tested 1) GSK and mTOR inhibitors which are known to support the maintenance and self-renewal of HSCs.

４．研究成果

To achieve efficient HDR-editing in HSCs, we optimized the genomed-editing condition in vitro and in vivo. We targeted the Ptprc (CD45) and quantified single amino acid polymorphism between CD45.1 and CD45.2 (Figure 1A) by flow cytometry and compared the edited (NHEJ-CD45.1-/-; CD45.2-/- or HDR-CD45.1+/+; CD45.2+/-) or non-edited (wild-type- CD45.2+/+) populations (Figure 1B). To optimize the editing condition, tested and compared electroporation and nucleofection with 80-160bp of single-strand DNA template. 140bp of single-strand DNA template with nucleofection showed the highest HDR-editing (Figure 1C). Further, improve HDR-mediated editing in LT-HSCs

GSK and mTOR inhibitors which are known to support the maintenance and self-renewal of HSCs. Treatment with those two inhibitors improved HDR-mediated editing from ~5% to 10-30% in vitro (Figure 1D); from ~1% to ~10% in vivo (Figure 2C).

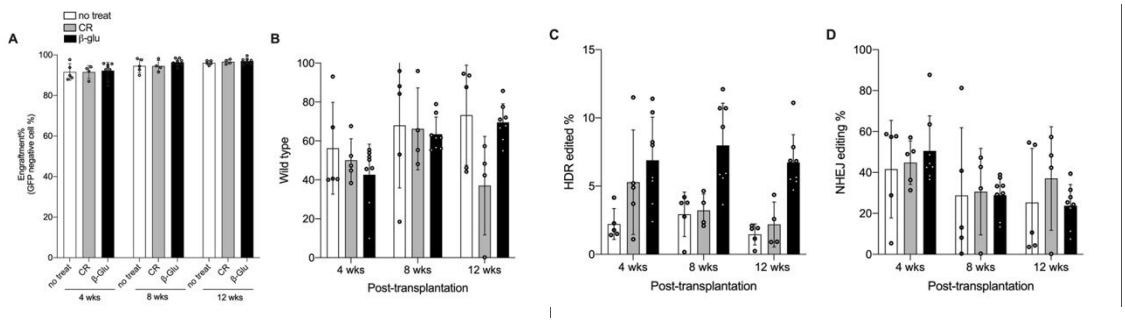

Figure 2. A. Engraftment of genome-edited/expanded HSCs after transplantation. **B.** Non-edited or wild type cell engraftment ratio in recipient **C.** HDR-edited cells engraftment ratio **D.** NHEJ-edited cells engraftment ratio after transplantation compared in non-treated, mTOR and GSK inhibitor (CR) treated and beta-glucan (β-glu) treated group.

Used the phosphonothioate-modified single-strand DNA template to conjugate the template to Cas9, and it showed 5-20% in vitro (Figure 1D); ~5% in vivo (Figure 2C) after serial transplantation, however, GSK and mTOR inhibitor treatment did not show the improved result of long-term repopulation in the recipient. Thus, we tested different strategy to improve genome-edited cell engraftment after transplantation by treating the cells with beta-glucan. Beta-glucan has previously been used to enhance the engraftment of hCD34+ transplantation. To test whether the beta-glucan improves the engraftment of genome-edited long-term HSCs, we treated the long-term HSCs with beta-glucan before the genome-editing procedure and transplanted them to the recipient. Our result showed more than 2 times engraftment ratio than non-treated cells after transplantation when we treat the cells with beta-glucan (Figure 2A-D). Further, beta-glucan treatment also improves myelopoiesis from HDR-edited cells, which compared with non-treated group (Figure 3A-B).

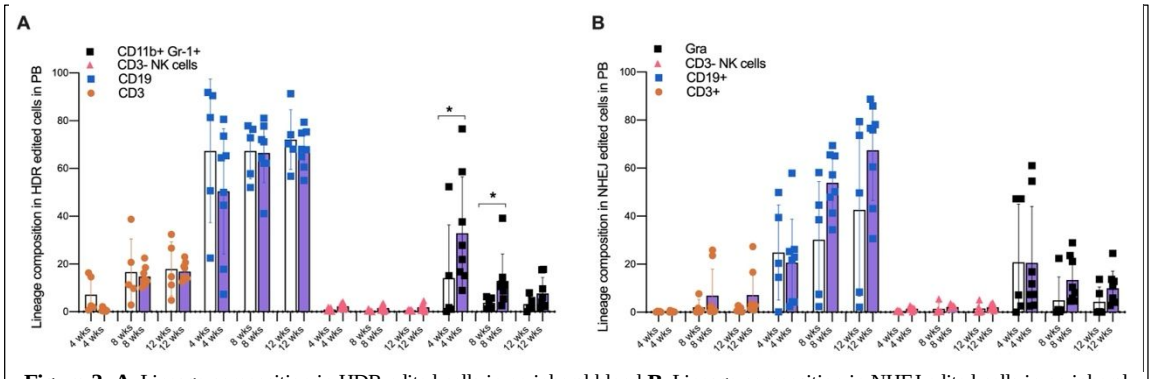

Figure 3. A. Lineage composition in HDR edited cells in peripheral blood **B.** Lineage composition in NHEJ edited cells in peripheral blood.

These results showed that ex vivo expanded, and HDR-edited cells are capable to repopulate HDR-edited multilineage cells after transplantation with beta-glucan treatment, demonstrated that precise genome editing of expanded long-term HSCs is feasible. The current study demonstrated that precise genome editing of expanded LT-HSCs is feasible, however, further, improvement is necessary for the realization of this method to cure genetic blood disorders.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　0件／うち国際共著　2件／うちオープンアクセス　0件）

１．著者名 Byambaa Suvd、Uosaki Hideki、Ohmori Tsukasa、Hara Hiromasa、Endo Hitoshi、Nureki Osamu、Hanazono Yutaka	４．巻 20
２．論文標題 Non-viral ex-vivo genome-editing in mouse bona fide hematopoietic stem cells with CRISPR/Cas9	５．発行年 2021年
３．雑誌名 Molecular Therapy - Methods & Clinical Development	６．最初と最後の頁 451～462
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.omtm.2021.01.001	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Hiromasa Hara, Natsagdorj Munkh-Erdene, Suvd Byambaa, Yutaka Hanazono	４．巻 69
２．論文標題 Nonviral Ex Vivo Genome Editing in Mouse Bona Fide Hematopoietic Stem Cells with CRISPR/Cas9	５．発行年 2023年
３．雑誌名 Genome Editing in Animals: Methods and Protocols	６．最初と最後の頁 213-221
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Suvd Byambaa
２．発表標題 Targeted genome-editing of murine hematopoietic stem cells by CRISPR/Cas9
３．学会等名 The 27th Annual Meeting of Japan Society of Gene and Cell Therapy
４．発表年 2022年

１．発表者名 Suvd Byambaa
２．発表標題 Targeted genome-editing of murine hematopoietic stem cells by CRISPR/Cas9
３．学会等名 The Molecular Biology Society of Japan
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

IMSUT joint research program
https://www.ims.u-tokyo.ac.jp/imsut/content/000006020.pdf

JICHI MEDICAL UNIVERSITY YOUNG INVESTIGATOR AWARD　2021

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関