



UK-5099, a mitochondrial pyruvate carrier inhibitor, recovers impaired neutrophil maturation caused by AK2 deficiency in human pluripotent stem cell models

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ABSTRACT

Reticular dysgenesis (RD) is a rare genetic disease caused by gene mutations in the ATP:AMP phosphotransferase, adenylate kinase 2 (AK2). Patients with RD suffer from severe combined immunodeficiency with neutrophil maturation arrest. Although hematopoietic stem cell transplantation can be a curative option, it is invasive, and complications of agranulocytosis-induced infection worsen the prognosis. Here, we report that the use of UK-5099, an inhibitor of the mitochondrial pyruvate carrier (MPC), on hemo-angiogenic progenitor cells (HAPCs) derived from AK2-deficient induced pluripotent stem cells improved neutrophil maturation. Reactive oxygen species (ROS) levels in AK2-deficient HAPCs remained unchanged throughout all experiments, implying that UK-5099 improved the phenotype without affecting ROS levels. Overall, our results suggest that the MPC is a potential therapeutic target for the treatment of neutrophil maturation defects in RD.

1. Introduction

Reticular dysgenesis (RD) is a rare genetic disease caused by loss-of-function mutations in the human adenylate kinase 2 (AK2) gene [1–3]. Patients with RD suffer from severe combined immunodeficiency, which is characterized by T and B lymphocyte deficiencies, and agranulocytosis caused by a distinctive neutrophil maturation defect [4]. If these patients do not undergo early hematopoietic stem cell transplantation to reconstitute their immune systems, they succumb to infectious diseases during infancy [5]. In addition to immune system-related symptoms, they also experience progressive sensorineural hearing loss, which continues to advance even after hematopoietic stem cell transplantation, profoundly affecting their quality of life and development.

The survival rate of patients with RD undergoing hematopoietic stem cell transplantation is reportedly less than 70% [4], and transplantation is often complicated by infections due to agranulocytosis. Agranulocytosis that accompanies RD is refractory to granulocyte colony-stimulating factor (G-CSF), rendering infections arising from this a significant issue [6]. Given that hematopoietic stem cell transplantation is invasive and that sensorineural hearing loss does not improve afterwards, there is a demand for new therapeutic approaches to treat RD.

AK2 belongs to the AK family consisting of nine members (AK1–AK9) located at various intracellular sites, such as the cytoplasm, mitochondria, and nucleus [7–9]. AKs constitute a shuttle system that supplies ATP from the mitochondria to other intracellular compartments for

Abbreviations: AK2, adenylate kinase 2; ESC, embryonic stem cell; G-CSF, granulocyte colony-stimulating factor; gRNA, guide RNA; HAPC, hemo-angiogenic progenitor cell; HDR, homology-directed repair; HRP, horseradish peroxidase; iPSC, induced pluripotent stem cell; KO, knockout; MPC, mitochondrial pyruvate carrier; NEM, N-ethylmaleimide; PSC, pluripotent stem cell; RD, reticular dysgenesis; ROS, reactive oxygen species.

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various energy-consuming cellular activities. They also regulate many biological processes by controlling AMP signaling [10,11]. AK2 is located in the intermembrane space of mitochondria and catalyzes a reversible reaction involving the phosphoryl transfer of adenine nucleotides [11]. Studies using patient-derived induced pluripotent stem cells (iPSCs) and zebrafish models have shown that AK2 deficiency leads to mitochondrial metabolic disorders, in turn causing oxidative stress that impairs the function of hematopoietic precursor and inner ear hair cells [12,13]. It was demonstrated that the addition of antioxidants can alleviate these effects [12,14]. However, they only partially recover phenotypes, and further recovery may require correcting the intracellular energy metabolic abnormalities caused by the AK2 mutation.

We have previously demonstrated that in hemo-angiogenic progenitor cells (HAPCs) derived from the iPSCs of patients with RD, there is an anomaly in the intracellular distribution of ATP, with an over-accumulation in mitochondria and inadequate supply to the nucleus [13]. One hypothesis for this phenomenon is that AK2 deficiency inhibits ATP efflux from mitochondria, leading to abnormal intracellular energy distribution and metabolic alterations. In this study, we used UK-5099, an inhibitor of the mitochondrial pyruvate carrier (MPC) protein, to inhibit the transport of pyruvate to mitochondria in HAPCs and enhance anaerobic glycolysis [15,16]. Using UK-5099, we aimed to elevate cytoplasmic and nuclear ATP concentrations, as well as mitochondrial ATP accumulation, through facilitating the transition to cytosolic glycolytic metabolism. We evaluated the effect of this intervention on the differentiation potential of HAPCs. To precisely evaluate this phenotype, we generated AK2-deficient pluripotent stem cell (PSC) lines via genome editing of wild-type iPSCs and embryonic stem cells (ESCs). Overall, our findings indicate that modifying the intracellular energy flux influences the granulocytic differentiation ability of hematopoietic precursor cells and suggest that the MPC represents a promising therapeutic target for RD.

2. Materials and methods

Ethical statement

Use of patient-derived iPSCs was approved by the Ethics Committee of Kyoto University (R0091), and written informed consent obtained from guardians of the patients with RD (ID: RD2, CIRA00067) in accordance with the Declaration of Helsinki. Use of human ESCs at Kyoto University was approved by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

2.1. Cell lines and culture

The human ESC line, KhES-1, was kindly provided by Dr. Hirofumi Suemori (Kyoto University, Kyoto, Japan) and the human iPSC line, 409B2, kindly provided by Dr. Keisuke Okita (Kyoto University). The patient-derived iPSC lines, RD and RD+AK2 iPSCs, were previously established [13].

PSCs were maintained on 6-well tissue culture plates coated with 0.25 $\mu\text{g}/\text{cm}^2$ Laminin511-E8 fragment iMatrix-511 silk (Nippi, Japan) with StemFit AK02 medium (Ajinomoto, Japan). PSCs were dissociated into single cells using $0.5 \times$ TrypLE Select (12563029; Thermo Fisher Scientific, USA) and 3000 cells plated in each well for passage. Thereafter, PSCs were treated with 10 μM Rock inhibitor Y-27632 (08945-71; Nacalai Tesque, Japan) for two days and switched to fresh AK02 medium without Y-27632 every two days.

2.2. Generation of homozygous AK2-knockout 409B2 and KhES-1 clones

We established AK2-knockout (KO) PSC clones by using the CRISPR-Cas9 system. The guide RNA (gRNA) sequence was designed based on the AK2 sequence in intron 3 and exon 4 (TTTA-CAGCTCGATGACCTCATGG). The cDNA encoding the gRNA sequence

was integrated into pUC118 DNA *HincII*/BAP vectors (3322; Takara Bio Inc., Japan). Cas9 and homology-directed repair (HDR) vectors (sc-404166 and sc-404166-HDR) were purchased from Santa Cruz Biotechnology (USA). Briefly, the HDR vector consisted of a cytomegalovirus promoter, red fluorescent protein, and puromycin resistance gene flanked by two loxP sites and homology arms. For targeting, PSCs were dissociated into single cells using $0.5 \times$ TrypLE Select. Next, gRNA, Cas9, and HDR vectors were introduced using a NEPA21 electroporator (Nepa Gene Co., Ltd., Japan). A total of 500,000 cells were used for each condition. The parameter settings of electroporation were as follows: voltage, 125 V; pulse length, 5 ms; pulse interval, 50 ms; number of pulses, 1; decay rate, 10%; polarity + as poring pulse and voltage, 20 V; pulse length, 50 ms; pulse interval, 50 ms; number of pulses, 5; decay rate, 40%; and polarity $\pm$ as transfer pulse.

After two days, cells were cultured with puromycin (1 $\mu\text{g}/\text{mL}$) for two days and subsequently with puromycin (5 $\mu\text{g}/\text{mL}$) for nine days. Surviving colonies were collected on day 11 after the start of selection. After genotyping, the loxP-flanked drug selection cassette was excised by expressing Cre recombinase, which was transfected using the FuGENE HD Transfection Reagent (E2311; Promega, USA). Cre with a nuclear localization signal (NLS-Cre) ORF was cloned into the multicloning site of a pLV-EF1a-MCS-IRES-RFP-Puro vector (cDNA-pLV09; Biosetti, USA) to express Cre recombinase in the nucleus.

For genotyping, genomic DNA was extracted using the PureLink Genomic DNA Kit (K1820-02; Life Technologies, USA). Exon 3, intron 3, and exon 4 of the AK2 gene were sequenced using a 3500xL Genetic sequencer (Applied Biosystems, USA). The primer sequences used for sequencing and PCR were as follows: Forward, 5'-AGTTGGGC-TAATGTCAGTCACC-3'; Reverse, 5'-GTGACATCCGCTGAAGTGGT-3'.

2.3. AK2 enzymatic activity

ATP:AMP phosphotransferase activity was analyzed using a coupled pyruvate kinase-lactate dehydrogenase assay, as previously described [17]. The reaction mixture (0.45 mL) contained 100 mM triethanolamine-HCl (pH 8.5), 75 mM KCl, 2.5 mM MgCl_2 , 1 mM AMP, 2.3 mM ATP, 0.4 mM NADH, 0.85 mM PEP, 2 U pyruvate kinase, and 2.75 U lactate dehydrogenase. AK2 activity was defined as the activity remaining after *N*-ethylemaleimide (NEM) treatment because AK1 is an SH enzyme that is inhibited by NEM, whereas AK2 is not. For NEM treatment, enzyme samples in 2 mM 3-(*n*-morpholino)propanesulfonic acid (pH 8.0) were incubated with 2 mM NEM for 1 h at 25 °C before the assay.

2.4. Hematopoietic cell differentiation from pluripotent stem cells

The hematopoietic cell differentiation protocol was slightly modified from that of a previously described serum- and feeder-free monolayer system [18]. G-CSF was removed from the cytokine cocktails. Briefly, undifferentiated PSC colonies were prepared on Laminin511-E8 fragment-coated culture plates with StemFit AK02 medium by seeding single cells onto 6-well plates. When individual colonies reached 700–1000 μm in diameter, the culture medium was replaced with Essential 8 medium (Thermo Fisher Scientific) containing 80 ng/mL BMP4 (R&D Systems, USA), 80 ng/mL VEGF (R&D Systems), and 4 μM GSK-3 inhibitor CHIR99021 (Merck Millipore). The cells were cultured at 37 °C, 5% CO_2 , and 5% O_2 during differentiation. On day two, the medium was replaced with Essential 6 medium (Thermo Fisher Scientific) containing 25 ng/mL bFGF (Wako, Japan), 80 ng/mL VEGF, 50 ng/mL SCF (R&D Systems), and 2 μM SB431542 (Sigma-Aldrich). On day four, the medium was replaced with StemPro34 medium (Life Technologies) containing 2 mM GlutaMAX (Invitrogen, USA), 80 ng/mL VEGF, 50 ng/mL SCF, 50 ng/mL Flt-3 Ligand (R&D Systems), 50 ng/mL IL-3 (R&D Systems), and 5 ng/mL thrombopoietin (R&D Systems). On days six and eight, the cytokines were switched to SCF, 50 ng/mL Flt-3 Ligand, 50 ng/mL IL-3, and 5 ng/mL thrombopoietin to differentiate the myeloid

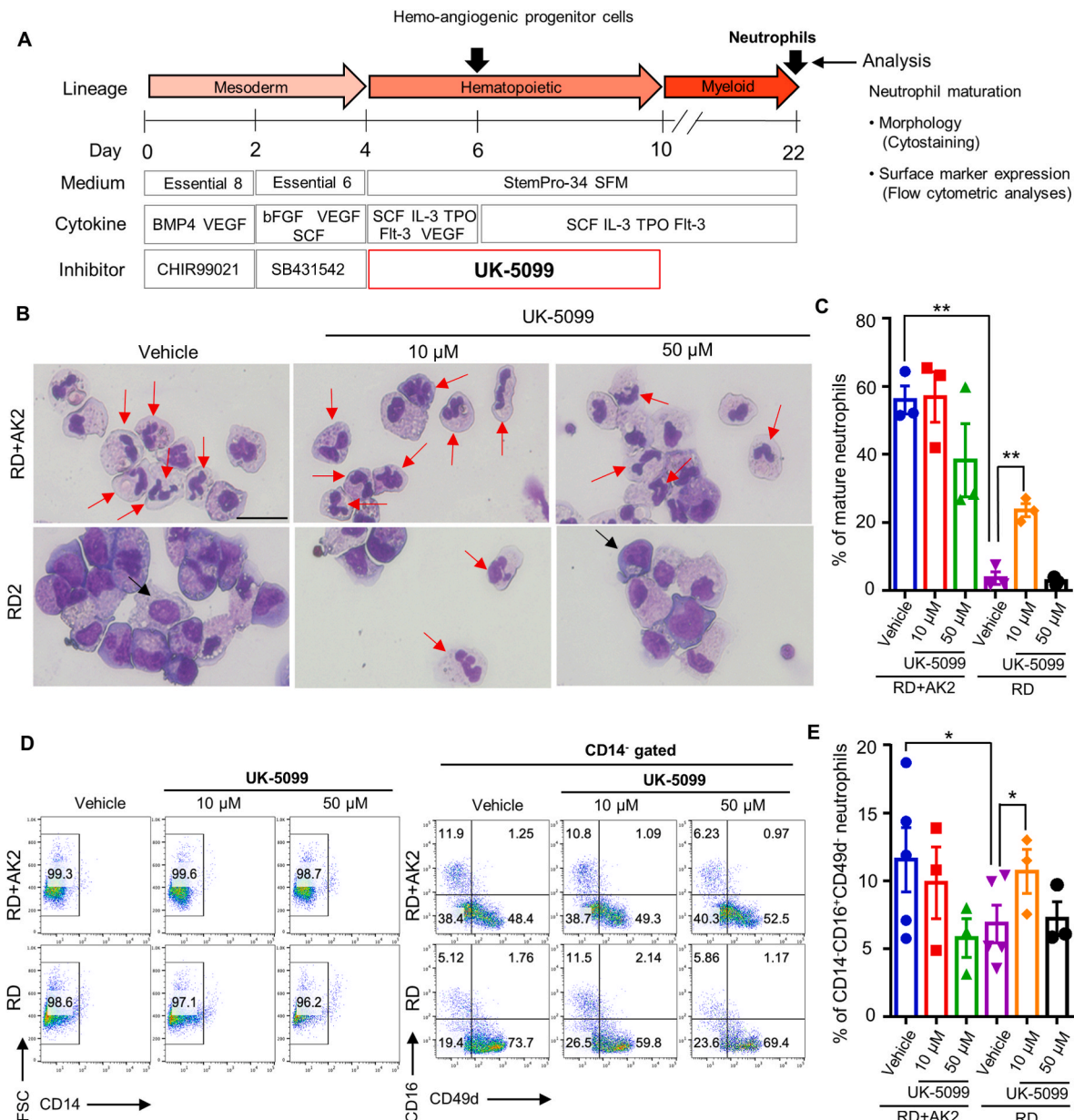


Fig. 1. UK-5099 improves neutrophil maturation in induced pluripotent stem cells derived from patients with reticular dysgenesis.

(A) A schematic diagram of hematopoietic differentiation. (B) Representative morphological appearance of neutrophils from induced pluripotent stem cells derived from patients with reticular dysgenesis (RD-iPSCs). Red and black arrows indicate mature and immature neutrophils, respectively. Scale bar = 25 μ m. (C) Quantification of mature neutrophils from RD and RD+AK2 iPSCs. (D) Representative flow diagrams of iPSC-derived myeloid cells to evaluate neutrophil maturation (day 22). (E) Quantification of CD14⁺CD16⁺CD49d⁺ mature neutrophils from RD and RD+AK2 iPSCs with UK-5099 treatment. (C, E) Data represent the mean $\pm$ SEM ($n = 3$ or 5 biologically independent experiments; * $p < 0.05$ and ** $p < 0.01$, two-tailed paired Student's t -test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

lineages. On day 10, 2 mL medium containing the same cytokines was added, and half of the medium containing the same cytokines replaced every three days until analysis. For UK-5099 treatment, 10 or 50 μ M UK-5099 in DMSO was added into medium from days 4–10 when replacing the medium.

2.5. Statistical analysis

All statistical analyses were performed using GraphPad Prism 6 software (GraphPad Soft, USA). Results are shown as the mean $\pm$ SEM and compared using one-way ANOVA followed by Tukey's multiple comparisons test, or two-tailed unpaired or paired Student's t -test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. UK-5099 improves neutrophil maturation in induced pluripotent stem cells derived from patients with reticular dysgenesis

Our previous work suggested that hematopoietic differentiation from iPSCs with AK2 disease-associated mutations was impaired from the CD34⁺KDR⁺ HAPC stage and onwards [13]. Therefore, we treated iPSCs derived from patients with RD (RD-iPSCs) with UK-5099 between days 4–10 of hematopoietic differentiation when HAPCs appeared in the culture (Fig. 1A). Morphologically, the frequency of mature neutrophils (segmented and band neutrophils) differentiated from RD-iPSCs was significantly impaired compared with those from the AK2-supplemented

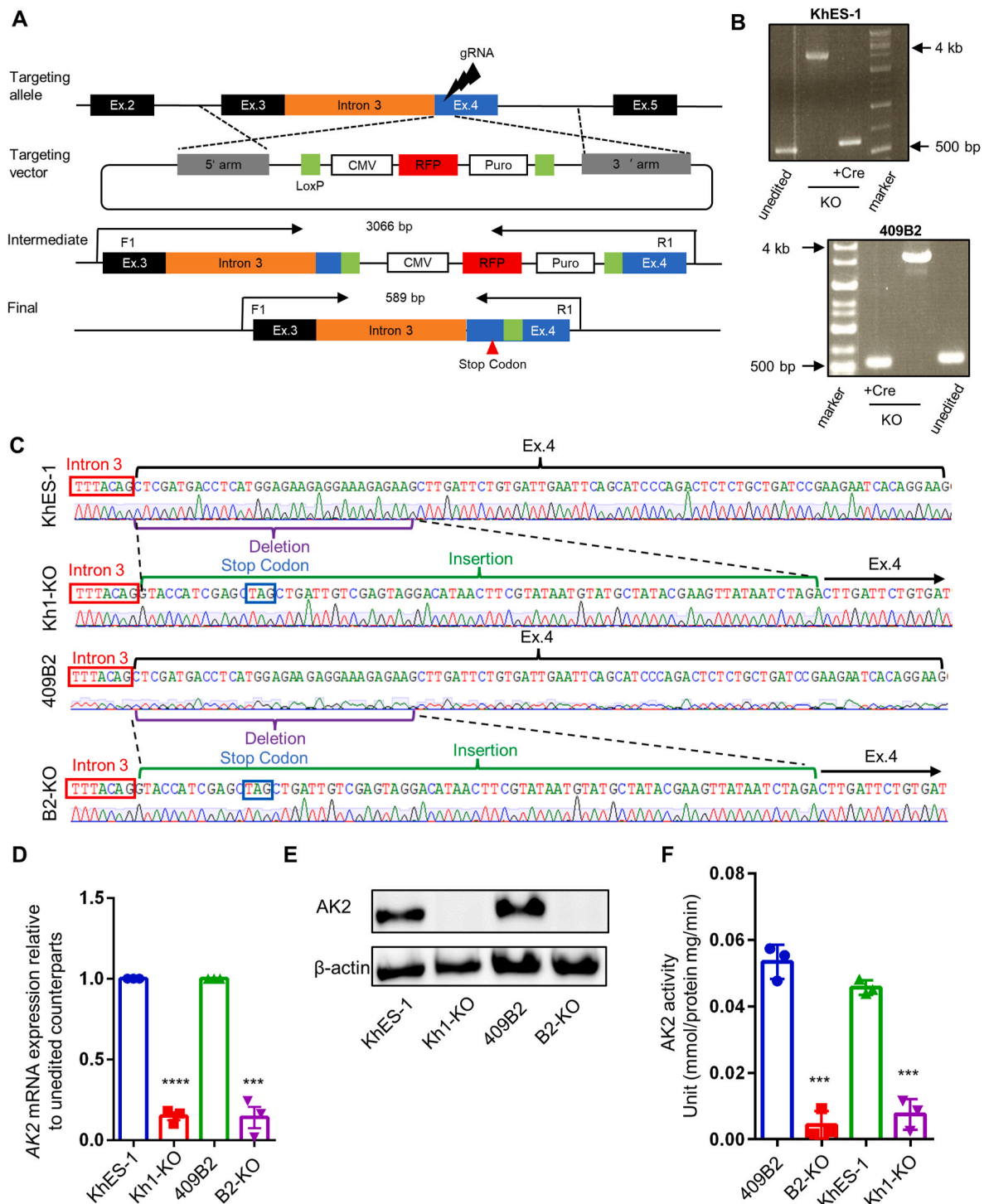


Fig. 2. Establishment of AK2-knockout pluripotent stem cell lines.

(A) Targeting strategy for knocking out the AK2 allele. F1, forward primer; R1, reverse primer. (B) Confirmation of the integration and excision of targeting vectors using genomic PCR. (C) Sanger sequencing of the AK2 allele after the excision of targeting vectors. Blue boxes indicate in-frame stop codons. (D) AK2 mRNA expression in undifferentiated pluripotent stem cells (PSCs). Relative values to wild-type counterparts are shown. (E) AK2 protein expression in undifferentiated PSCs. (F) AK2 enzymatic activity in undifferentiated PSCs. (D, F) Data represent the mean $\pm$ SEM ($n = 3$, biologically independent experiments; *** $p < 0.001$ and **** $p < 0.0001$, unpaired Student's t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

isogenic counterpart (RD+AK2-iPSCs) (Fig. 1B and C, Supplementary Fig. 1). Neutrophil maturation from RD-iPSCs was significantly improved after 10 μ M UK-5099 treatment (Fig. 1B and C, Supplementary Fig. 1). In contrast, UK-5099 showed no further synergetic effect with AK2 supplementation and decreased at higher concentrations (50 μ M

(Fig. 1B and C). We then evaluated the expression of neutrophil surface antigens. CD16⁺CD49d⁺ fractions were defined as mature neutrophils after excluding monocytic cells with CD14-negative gating [19]. As expected, the percentage of CD14⁺CD16⁺CD49d⁺ mature neutrophils was significantly higher in RD+AK2 iPSC-derived hematopoietic cells

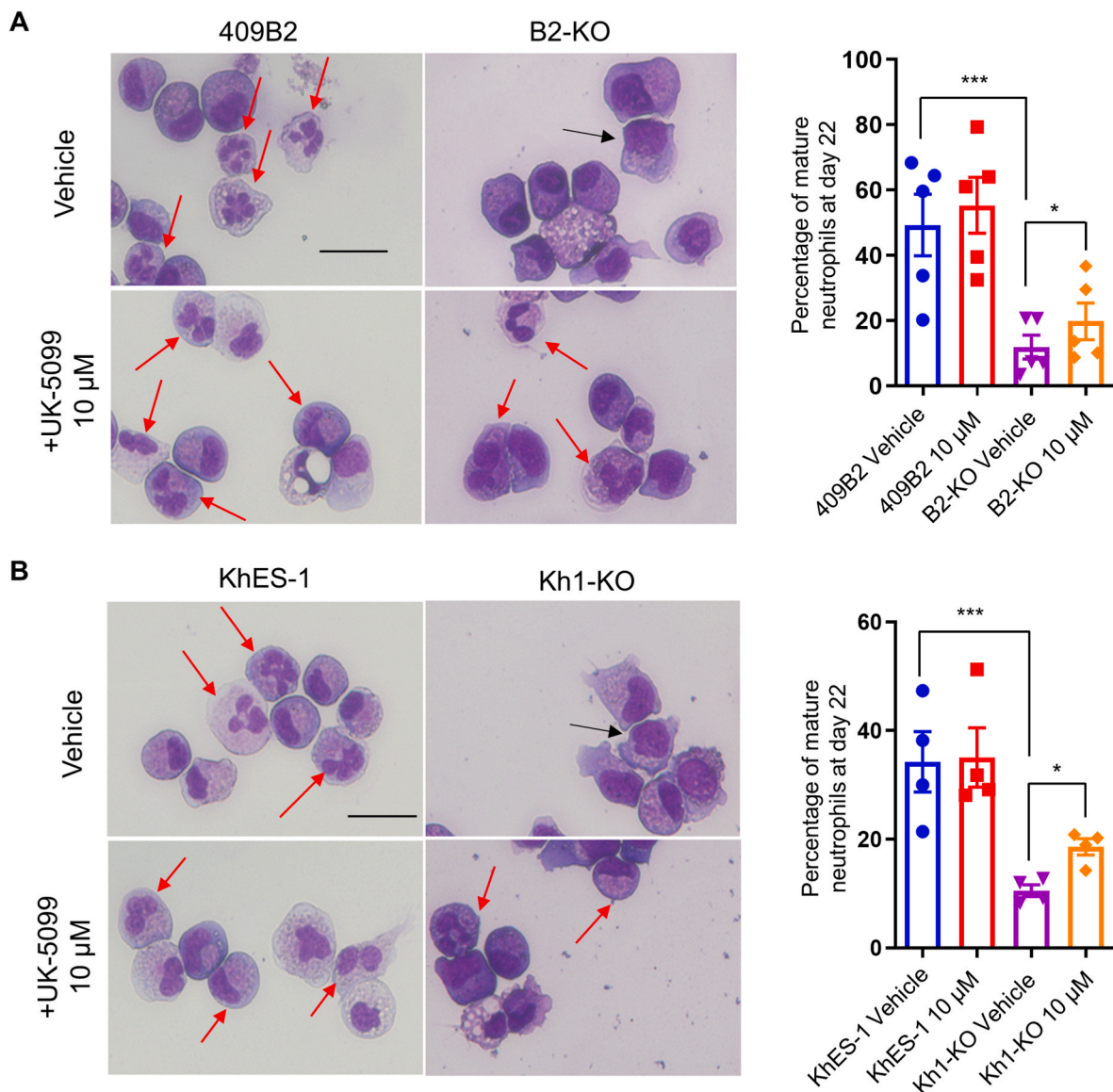


Fig. 3. UK-5099 improves neutrophil morphological maturation from AK2-knockout pluripotent stem cells.

(A) Representative morphological appearance of neutrophils from 409B2 induced pluripotent stem cells (iPSCs) at day 22 (left). Quantification of mature neutrophils from 409B2 iPSCs (right). (B) Representative morphological appearance of neutrophils from KhES-1 embryonic stem cells (ESCs) at day 22 (left). Quantification of mature neutrophils from KhES-1 ESCs (right). (A, B) Data represent the mean $\pm$ SEM ($n = 5$ (A) or 4 (B) biologically independent experiments; $*p < 0.05$ and $***p < 0.001$, two-tailed paired Student's t -test).

than in RD-iPSCs (Fig. 1D and E). When HAPCs were treated with 10 μ M UK-5099, the output of CD14⁺CD16⁺CD49d⁺ mature neutrophils from RD-iPSCs was significantly higher than that from untreated cells, whereas no significant difference between treated and untreated RD+AK2 iPSC-derived HAPCs was observed (Fig. 1D and E). To confirm the correlation between surface markers and cell morphology, we sorted out CD14⁺CD16⁺CD49d⁺ and CD14⁺CD16⁺CD49d⁺ fractions differentiated from control PSC lines (409B2 iPSCs and KhES-1 ESCs). While the CD14⁺CD16⁺CD49d⁺ fractions contained mature neutrophil-like cells with segmented nuclei and cytoplasmic granules, the CD14⁺CD16⁺CD49d⁺ fractions contained more immature cells with round nuclei and an azurophilic cytoplasm, confirming that fractionation by surface markers correlated with morphological evaluations (Supplementary Fig. 2). Overall, UK-5099 treatment of HAPCs improved neutrophil maturation from RD-iPSCs.

3.2. Establishment of AK2-knockout pluripotent stem cell lines

The previous experiments compared phenotypes of iPSC pairs consisting of RD-iPSCs and RD+AK2 iPSCs in which AK2 was super-physiologically expressed. However, for accurate phenotypic evaluations, AK2-deficient PSC clones must be compared with PSC clones with physiological AK2 expression in an isogenic background. Thus, we generated homozygous AK2-KO lines from wild-type PSC lines (409B2 iPSCs and KhES-1 ESCs) using the CRISPR-Cas9 system (hereafter referred to as B2-KO and Kh1-KO, respectively; Fig. 2A). Integrated targeting vectors were removed by introducing Cre recombinase (Fig. 2B and Supplementary Fig. 3A). Sanger sequencing confirmed the insertion of an in-frame stop codon in exon 4 of AK2 (Fig. 2C). AK2 mRNA expression was significantly decreased in both AK2-KO PSC lines (Fig. 2D), resulting in the loss of full-length AK2 protein production (Fig. 2E and Supplementary Fig. 3B). Consistent with this finding, AK2 enzymatic activity in AK2-KO PSCs was almost completely abrogated (Fig. 2F). We confirmed that the AK2-KO PSC clones maintained a

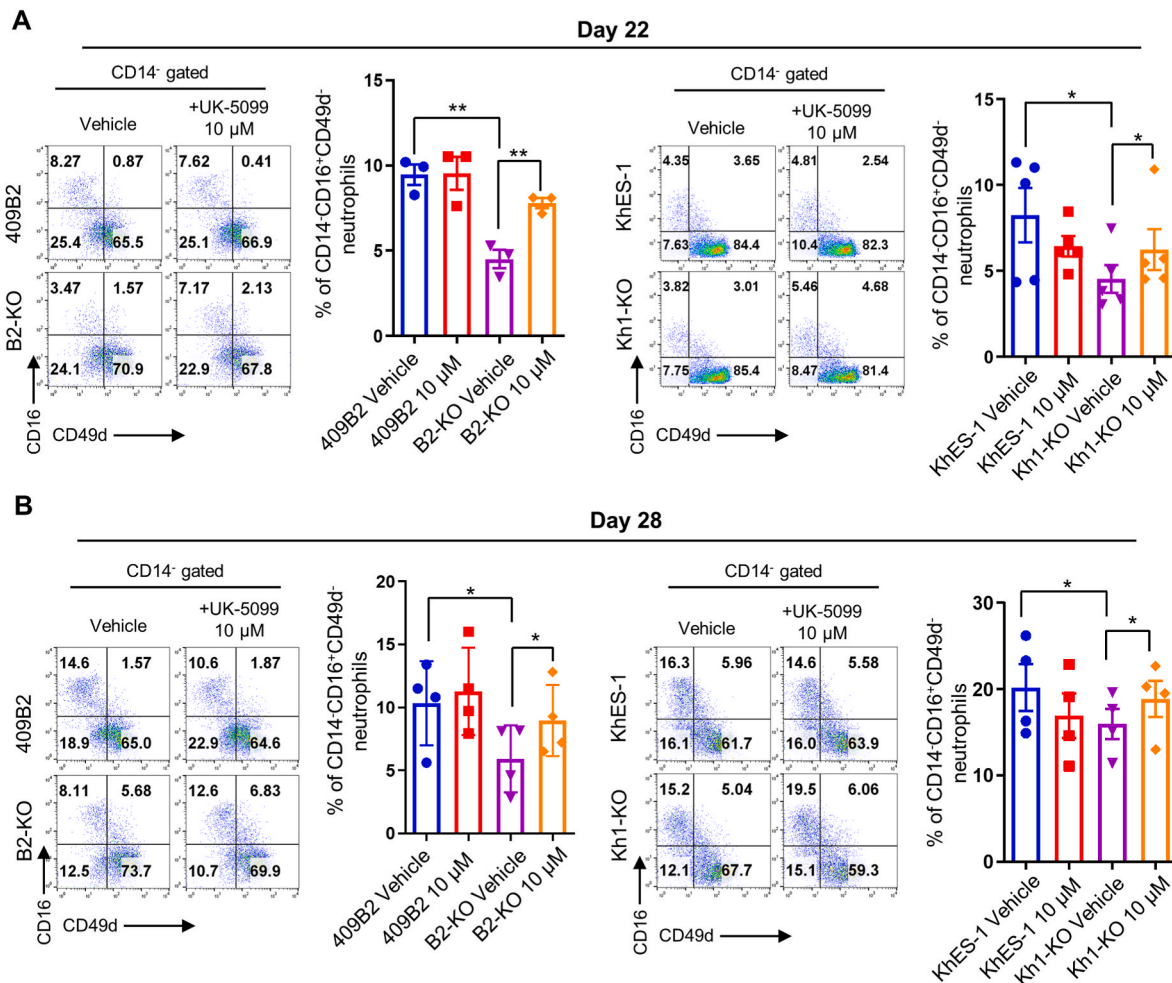


Fig. 4. UK-5099 recovers mature neutrophil-specific surface marker expression on AK2-knockout neutrophils.

(A, B) Representative flow diagrams of pluripotent stem cell (PSC)-derived myeloid cells to evaluate neutrophil maturation and quantification of CD14⁺CD16⁺CD49d⁺ mature neutrophils from PSCs (bar graph) at days 22 (A) and 28 (B). Data represent the mean $\pm$ SEM ($n = 3-5$ biologically independent experiments; * $p < 0.05$ and ** $p < 0.01$, two-tailed paired Student's t -test).

normal karyotype (Supplementary Fig. 3C).

3.3. UK-5099 improves neutrophil maturation from AK2-knockout pluripotent stem cells

AK2-KO PSC lines were differentiated into neutrophils to determine whether they recapitulated neutrophil maturation arrest, similar to that of patient-derived iPSCs. In both PSC pairs, the KO clones yielded a significantly lower frequency of morphologically mature neutrophils than that of their unedited counterparts (Fig. 3A and B, Supplementary Fig. 4). Consistent with this, the frequency of CD14⁺CD16⁺CD49d⁺ mature neutrophils was significantly decreased in AK2-KO PSC lines (Fig. 4A and Supplementary Fig. 5A). Therefore, AK2-KO PSC clones successfully phenocopied the *in vitro* neutrophil maturation arrest observed in RD-iPSCs.

We then evaluated the effect of UK-5099 on neutrophil maturation differentiated from AK2-KO PSCs. In the following experiments, 10 μ M UK-5099 was added, which showed improvement of neutrophil differentiation in RD-iPSCs (Fig. 1). Similar to that of RD-iPSCs, UK-5099 treatment of HAPCs derived from KO PSCs significantly improved the morphological maturation of neutrophils (Fig. 3A and B, Supplementary Fig. 4) and the frequency of CD14⁺CD16⁺CD49d⁺ mature neutrophil output (Fig. 4A and Supplementary Fig. 5A) compared with those of untreated cells. Notably, consistent differences in the frequency of CD14⁺CD16⁺CD49d⁺ on day 28 further demonstrates the dependability

of these markers in our differentiation system when using UK-5099 (Fig. 4B and Supplementary Fig. 5B). Overall, UK-5099 improved neutrophil differentiation, even in the PSCs in which AK2 enzyme activity was eliminated.

3.4. UK-5099 does not alter intracellular reactive oxygen species status

Oxidative stress is increased in hematopoietic cells of AK2 mutant zebrafish, and antioxidant treatment rescues hematopoietic differentiation defects both *in vivo* and *ex vivo* [12]. In contrast UK-5099 upregulated ROS production in the prostatic cancer cell line LnCap [15]. Collectively, we hypothesized that the restoration of differentiation defects by UK-5099 is associated with alterations in ROS levels in HAPCs. Evaluation of ROS levels revealed no obvious differences between unedited and AK2-KO HAPCs, which UK-5099 treatment also did not affect (Supplementary Figs. 6A and 6B). Therefore, the improvement in neutrophil maturation after UK-5099 treatment was not mediated by modulating ROS status.

4. Discussion

In this study, we demonstrated that terminal differentiation into neutrophils could be improved by adding an MPC inhibitor to HAPCs derived from AK2-mutant or -KO PSCs. In our previous studies, we compared RD-iPSCs and AK2-overexpressed RD-iPSCs, in which non-

physiological AK2 expression in AK2-complemented iPSCs resulted in phenotypic differences [13]. Therefore, in this study, we established precise isogenic PSC pairs by knocking out AK2 in wild-type PSCs and performed quantitative phenotypic comparisons.

Interestingly, the effects of UK-5099 were observed only in AK2-KO or -mutated PSCs and not in the wild-type. However, the phenotypic improvement induced by UK-5099 was insufficient, particularly in AK2-KO cells. This could be because the administration period of the compound was not optimized. Alternatively, a complex mechanism involving various factors, such as oxidative stress, as reported in other studies, may also be involved [12].

Mammalian cells predominantly use two sequential carbon metabolic pathways, glycolysis in the cytoplasm and oxidative phosphorylation in the mitochondria, to generate ATP as an energy source for various cellular activities. Depending on the cell type, either one or a combination of these metabolic pathways is required in different proportions [20]. Notably, hematopoietic stem cells gradually transition their energy metabolism from anaerobic glycolysis to oxidative phosphorylation during hematopoietic differentiation [21]. Based on our previous findings [13], we employed UK-5099 to alter intracellular energy flux and distribution. Given that AK2 deficiency may induce the abnormal formation of proton concentration gradients in mitochondria, blocking the influx of pyruvate into the TCA cycle with UK-5099 may have prevented further supply of proton substrates for unfavorable metabolic chain reactions. However, whether UK-5099 improves neutrophil differentiation via this mechanism remains unclear, implying the need to consider other potential mechanisms.

We found no significant difference in ROS production between unedited and AK2-KO HAPCs, in contrast to previous reports suggesting elevated ROS levels in both hematopoietic tissues and sensory organs of AK2-mutant zebrafish [12,14]. As G-CSF was not used for neutrophil differentiation, it is plausible that it might be a critical factor accounting for the discrepancy in ROS production observed. Several studies have demonstrated that G-CSF treatment augments ROS production in mouse hematopoietic progenitor cells [22–24]. Thus, it is conceivable that G-CSF may specifically enhance ROS production in AK2-KO HAPCs. However, our findings show that treatment with UK-5099 did not reduce ROS levels in either unedited or KO HAPCs, suggesting that the antioxidant effect may not be the primary factor contributing to the observed rescue phenotype of neutrophil differentiation.

Our study provides evidence that UK-5099 enhances neutrophil maturation in AK2-deficient or -KO iPSCs. Given the crucial role of neutrophils in the immune response, these findings may have significant implications for therapeutic strategies targeting conditions characterized by neutrophil deficiency, such as RD. Thus, future research should focus on elucidating the mechanism by which UK-5099 links metabolic distribution and differentiation programs, particularly in the absence of AK2. The effects of lymphocyte deficiency, another key manifestation of RD, should also be assessed. Moreover, investigation of *in vivo* disease models in combination with the potential side effects and toxicity of UK-5099 are required due to the lower number of mature neutrophils detected at high concentrations. Regardless of these challenges, UK-5099 and the MPC may present viable therapeutic targets for the neutrophil maturation defects observed in RD.

CRedit authorship contribution statement

Jingxin Wang: Writing – original draft, Visualization, Validation, Investigation. **Norikazu Saiki:** Visualization, Validation, Resources, Investigation, Conceptualization. **Ayako Tanimura:** Visualization, Validation, Investigation. **Takafumi Noma:** Validation, Data curation. **Akira Niwa:** Supervision, Data curation. **Tastutoshi Nakahata:** Validation, Data curation. **Megumu K. Saito:** Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Megumu K. Saito reports financial support was provided by Japan Agency for Medical Research and Development. Megumu K. Saito reports financial support was provided by Terumo Life Science Foundation. Megumu K. Saito reports financial support was provided by iPS Cell Research Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2023.149211>.

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