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Plasminogen activator inhibitor type-1 is a negative regulator of hematopoietic regeneration in the adipocyte-rich bone marrow microenvironment

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ABSTRACT

Bone marrow adipocytes (BMAs) have recently been recognized as a niche component with a suppressive function. Obese individuals with abundant BMAs exhibit impaired hematopoietic regeneration after hematopoietic stem cell transplantation (HSCT). We hypothesized that plasminogen activator inhibitor type-1 (PAI-1), an adipokine that regulates the fibrinolytic system, contributes to impaired hematopoiesis in bone marrow (BM) microenvironment with abundant BMAs. We demonstrated that BMAs differentiated *in vitro* could secrete PAI-1 and were positive for PAI-1 *in vivo*. In addition, the abundance of BMAs was associated with high levels of PAI-1 expression. The BMA-rich microenvironment exhibited impaired hematopoietic regeneration after HSCT when compared with a BMA-less microenvironment. The impaired hematopoietic regeneration in BMA-rich microenvironment was significantly alleviated by PAI-1 knockout or PAI-1 inhibitor treatment. Obese mice with abundant BMAs, compared with normal-weight mice, exhibited higher bone marrow PAI-1 concentrations, increased fibrinolytic system suppression, and lower stem cell factor (SCF) concentrations after HSCT. PAI-1 inhibitor administration significantly activated the fibrinolytic system in obese mice, contributing to the higher SCF concentration. Moreover, PAI-1 inhibitor treatment significantly alleviated the impaired hematopoietic regeneration in obese mice both after 5-fluorouracil injection and HSCT. These results indicate that PAI-1 hinders hematopoietic regeneration in BMA-rich microenvironments. The blockade of PAI-1 activity could be a novel therapeutic means of facilitating hematopoietic reconstitution in BMA-rich patients.

1. Introduction

Obesity is highly prevalent worldwide and is associated with several complications such as cardiovascular diseases [1]. Obesity-associated inflammation is caused by proinflammatory cytokines, or adipokines, which are produced in adipose tissues. This condition leads to insulin resistance and atherosclerosis [2]. Adipokines are thought to be secreted mainly by visceral adipose tissues [2]. The bone marrow (BM) is the site of hematopoietic cell production. However, it is also a site of adipocyte accumulation in obese individuals; the resulting “yellow” BM contains abundant BM adipocytes (BMAs) [3,4] that can cause diseases, such as osteoporosis [4]. Obese individuals also exhibit hematopoietic impairments such as myelopoiesis and monocytosis [5,6], which contribute to the development of atherosclerosis [2]. Taken together, both BMAs and visceral adipose tissues may be associated with the abnormal metabolic and inflammatory characteristics observed in obese individuals, especially hematopoietic impairment.

Hematopoietic stem cells (HSCs) reside in specialized BM microenvironments, and the components of these niches regenerate hematopoietic cell populations by precisely regulating HSC self-renewal, differentiation, and retention [7]. These complex and heterogenic components include osteoblasts, endothelial cells, and BM stromal cells (BMSCs) [7]. BMAs differentiate from Lepr^{+} BMSCs

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via adipogenic progenitors and precursors [8]. Recently, these cells have been recognized as a component of the functional niche component and regulator of hematopoiesis [9]. A decade ago, Naveiras et al. identified BMAs as negative regulators of hematopoietic regeneration [10] through an experiment wherein A-ZIP/F fatless mice with suppressed adipogenesis exhibited accelerated hematopoietic progenitor expansion and post-transplant hematopoietic reconstitution after hematopoietic stem cell transplantation (HSCT). Several other studies also reported the inhibitory effects of BMAs on hematopoietic regeneration [3,4,11,12]. A negative correlation between the numbers of BMAs and HSCs was also reported in a human clinical study [13]; however, the mechanisms underlying the suppressive biological effects of BMAs on hematopoiesis remain to be elucidated.

Plasminogen activator inhibitor type-1 (PAI-1), a well-known adipokine, is a negative regulator of the fibrinolytic system [14]. PAI-1 inhibits the serine protease activity of urokinase or tissue plasminogen activator, which proteolytically converts plasminogen into the active enzyme plasmin and thus inhibits the fibrinolytic system. Heissig et al. previously reported that in the BM, activation of the fibrinolytic system gives rise to the matrix metalloproteinase-mediated release of stem cell factor (SCF) from BMSCs [15], elucidating the involvement of the BM fibrinolytic system in hematopoiesis. Subsequently, we demonstrated that PAI-1 is a negative regulator of the fibrinolytic system in BM and acts as an inhibitor of hematopoietic regeneration [16]. After myeloablative irradiation, the concentration of PAI-1 increases considerably in the hypocellular BM [16], which contains abundant BMAs. The BMA-rich microenvironment harbored by obese individuals may be related to the observed higher PAI-1 concentrations in this population [17]. Therefore, we hypothesized that PAI-1 produced by BMAs would be associated with the impaired hematopoietic regeneration observed in BMA-rich microenvironments.

In this study, we compared the properties of hematopoietic regeneration between BMA-rich tail vertebrae versus BMA-less femurs and BMA-rich obese versus BMA-less normal-weight mice. We demonstrated that PAI-1 hindered hematopoietic regeneration in BMA-rich microenvironments, and that the blockade of PAI-1 activity facilitated hematopoietic regeneration after HSCT in BMA-rich recipients.

2. Materials and methods

2.1. Mice

Eight- to 12-week-old C57BL/6J and PAI-1 KO mice were purchased from Charles River Laboratory (Yokohama, Japan, www.crj.co.jp) and the Jackson Laboratory (Bar Harbor, ME, USA, www.jax.org). Diet-induced obese (DIO) C57B6/J mice were fed a 60 kcal% fat diet (D12492, Research Diets Inc.) for 12 weeks prior to starting the experiments, beginning at 4 weeks of age. Normal-weight mice were fed a control diet (CE-2 [12 kcal% fat], Clea Japan, www.clea-japan.com). Food was freely available. All mice were housed in cages at the animal facility of Tokai University School of Medicine and were treated in accordance with the institutional guidelines. The Institutional Review Board of Tokai University School of Medicine approved all animal experimental protocols.

2.2. Cell transplantation and PAI-1 inhibitor administration

Recipient mice (Ly5.2) were lethally irradiated (9 Gy) in an X-ray irradiator (MBR-1520R-3, Hitachi Medico, Tokyo, Japan, www.hitachi-power-solution.com) and administered 3×10^6 Ly5.1 congenic BM mononuclear cells via tail vein injection. After transplantation, the mice were euthanized at the designated time points. To evaluate hematopoietic reconstitution after treatment with 5-fluorouracil (FU), this drug was injected intraperitoneally (150 mg/kg, Wako). In the experiments involving a PAI-1 inhibitor, either TM5614 (a specific PAI-1 inhibitor with an IC_{50} value of <6.95 μM in a tPA- dependent hydrolysis assay [18,19]) or vehicle (distilled water) was administered daily via a feeding needle and oral gavage for 14 consecutive days. Additional information for details of materials and methods are described in supplementary information.

2.3. Statistical analysis

At least 2 individual experiments were performed unless stated otherwise. Where applicable, data are presented as means ± standard errors and analyzed using Student's t-test or a one-way ANOVA adjusted using the Bonferroni method as appropriate. All *P* values were two-sided and considered to be significant at values of <0.05. The statistical analyses were performed using IBM SPSS Statistics, ver. 16.

3. Results

3.1. Elevated PAI-1 production in BMAs relative to other cell populations

PAI-1 is known to be produced in the visceral adipose tissue [17]. We found that differentiated adipocytic 3T3-L1 cells from an embryonic fibroblast line produced greater amounts of PAI-1 than undifferentiated cells (Fig. 1a). In contrast, no published studies have explored whether "bone marrow" adipocytes also secrete PAI-1. Thus, we first evaluated PAI-1 production in cultured BMAs differentiated from BMSC lines and confirmed that differentiated adipocytic OP9 cells also produced greater amounts of PAI-1 (Fig. 1a). These results suggest that adipocytes differentiated from BMSCs could also secrete PAI-1.

Next, we examined the expression of PAI-1 in the BM microenvironment *in vivo*. As previously reported [10], the BM microenvironment in the tail vertebrae was mainly consisted of perilipin-positive BMAs when compared with femurs (Fig. 1b). Remarkably, immunofluorescent staining demonstrated that these perilipin-positive BMAs were also positive for PAI-1 (Fig. 1c). A rare fraction of the $CD45^-$ $CD31^-$ $Ter119^-$ $Sca1^+$ $CD24^-$ $CD140b^+$ cells in the BM represents the adipocyte-progenitor cells [4,20]. Thus, we evaluated the percentage of the adipocyte-progenitor cells by flow cytometry and found that the former bones contained a significantly higher frequency of the adipocyte-progenitor cells (Fig. S1a). Furthermore, the expression of *Pai1* as well as other adipokines in the BM microenvironment was significantly higher in the tail vertebrae than in the femur (Fig. 1d). In the flow cytometry analysis, the mean fluorescence intensity of PAI-1 expression was higher in the adipocyte-progenitor cells than in the osteoblasts ($CD45^-$ $CD31^-$ $Ter119^-$ $Sca1^-$ cells) or other cells ($CD45^+$ and/or $CD31^+$ and/or $Ter119^+$ and $Sca1^-$ cells) (Fig. 1e). These results indicate that the abundance of BMAs in the BM microenvironment was associated with high levels of PAI-1 expression.

3.2. Hematopoietic regeneration after cell transplantation is impaired in the BMA-rich microenvironment

To examine whether BMAs would negatively affect hematopoietic reconstitution, lethally irradiated CD45.2 wild-type (WT) mice received hematopoietic cell transplants from a congenic CD45.1 donor (Fig. 2a). As expected, the hematopoietic regeneration was significantly less efficient in the BMA-rich tail vertebrae than in the BMA-less femurs (Fig. S2a). The percent of total donor

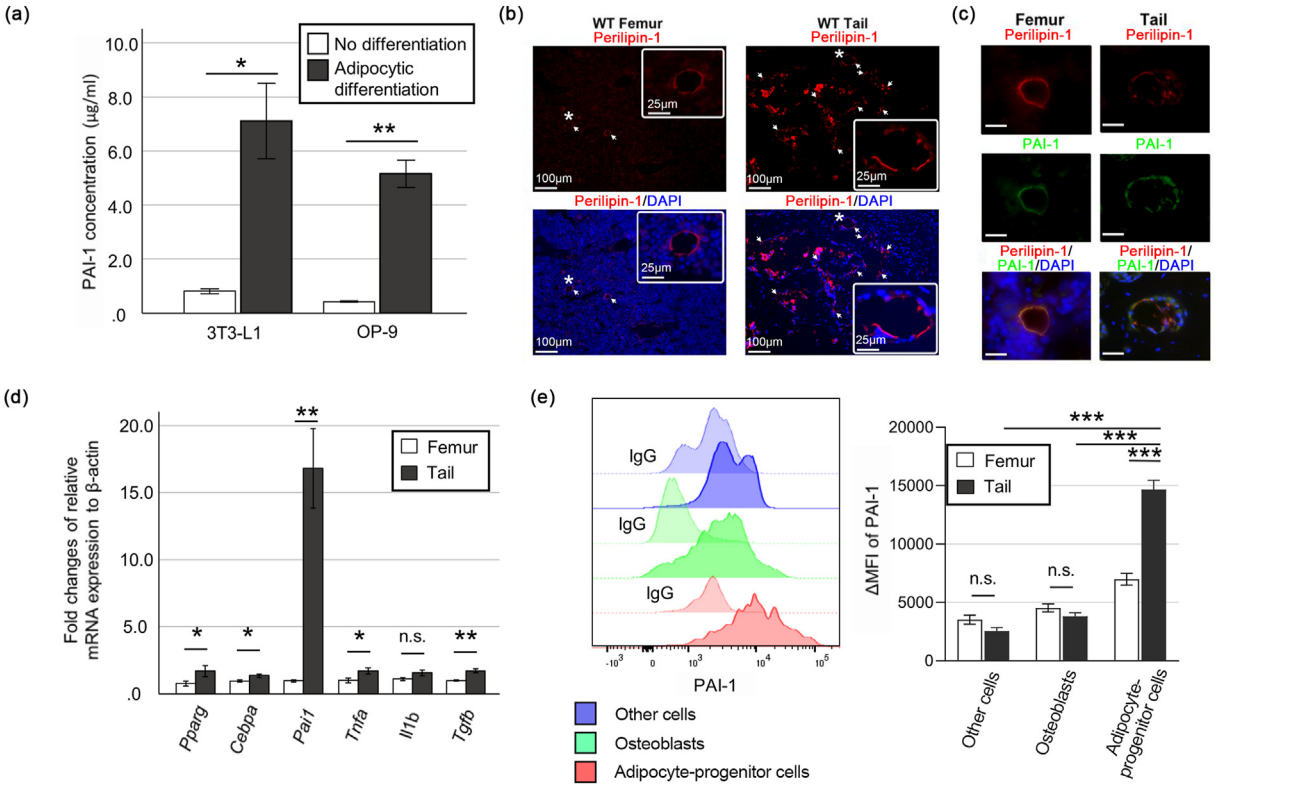

Fig. 1. Bone marrow adipocytes (BMAs) produce plasminogen activator inhibitor type-1 (PAI-1). (a): Comparison of plasminogen activator inhibitor type-1 (PAI-1) production by differentiated and undifferentiated OP9 or 3T3-L1 cell lines (n = 3 per group). (b, c): Representative images of BM sections of femurs and tail vertebrae from 8-weeks-old mice. BM sections were stained with anti-perillipin-1 (red) and anti-PAI-1 antibodies (green) and DAPI (blue). Arrows indicate perilipin-1-positive BMAs. Inserts are the higher magnification of the perilipin-1-positive BMAs (asterisk). Bars represent 100 μm and 25 μm (b) and 20 μm (c). (d): qRT-PCR analysis of the expression of genes encoding adipokines and adipogenic transcription factors in CD45$^-$CD31$^-$Ter119$^-$ BM microenvironment cells from the tail vertebrae relative to the expression in femoral cells (n = 4 per group). (e): Representative mean fluorescence intensities of PAI-1 expression in adipocyte-progenitor cells, osteoblasts, and other cells collected via enzymatic digestion (n = 6 per group). The Δfluorescence intensities were calculated as the difference between that of designated fractions stained with a PAI-1 antibody and isotype control. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

cell counts at days 2 and 7, relative to the counts at the steady state (day 0), were significantly lower in tail vertebrae than in the femurs (Fig. S2a). These data indicate that BMA-rich microenvironments exhibit impaired hematopoietic regeneration when compared with BMA-less microenvironments, which is consistent with the results of a previous study [10].

3.3. The suppressive effects of PAI-1 on hematopoietic regeneration after cell transplantation are larger in the BMA-rich microenvironment

After myeloablative irradiation, the proliferation of BMAs can be observed even in femur of the adipocyte-less microenvironment (Fig. S2b). In addition, we previously reported about the suppressive effects of PAI-1 on early-stage hematopoietic regeneration [16]. Considering the production of PAI-1 by BMAs and the impaired hematopoietic regeneration in the BMA-rich microenvironment, we hypothesized that PAI-1 inhibition after hematopoietic cell transplantation would have larger effects in the BMA-rich microenvironment. Therefore, we performed congenic cell transplantation using CD45.1 donor and CD45.2 PAI-1 knockout (KO) recipient mice and compared the hematopoietic regeneration between different bones (Fig. 2a). PAI-1 KO mice, in comparison with WT mice, exhibited the superior hematopoietic regeneration both in tail vertebrae and femurs (Fig. 2b). Remarkably, the relative increase in total donor cell and donor CD34$^-$Lineage$^-$c-kit$^+$Sca-1$^+$ HSC count for PAI-1 KO mice was more evident in tail vertebrae (Fig. 2b and c). This accelerated hematopoietic regeneration of PAI-1 KO mice in tail vertebrae was maintained even 4 weeks after transplantation (Figs. S2c and d), demonstrating the larger effects of PAI-1 knockout on hematopoietic regeneration in tail vertebrae than in femurs. Taken together, these results indicate that PAI-1 negatively regulates hematopoietic regeneration in the adipocyte-rich BM microenvironments.

3.4. PAI-1 inhibitor treatment effectively alleviates the impaired hematopoietic regeneration in the BMA-rich microenvironment

Next, we evaluated the effects of a PAI-1 inhibitor, TM5614, on hematopoietic regeneration in femurs and tail vertebrae (Fig. 2d). As observed in PAI-1 KO mice, TM5614-treated mice exhibited the superior hematopoietic regeneration both in femurs and tail vertebrae (Fig. 2e). Notably, the relative increase in total donor cell and donor HSC count for TM5614-treated mice was more evident in tail vertebrae (Fig. 2e and f), also indicating the larger effects of PAI-1 knockout on hematopoietic regeneration in tail vertebrae than in femurs. The ability of PAI-1 inhibition to facilitate hematopoietic regeneration in the tail vertebrae was also maintained 4 weeks after transplantation (Figs. S2e and f). These results indicate that PAI-1 inhibition can alleviate impaired hematopoietic regeneration after cell transplantation, especially in BMA-rich microenvironments.

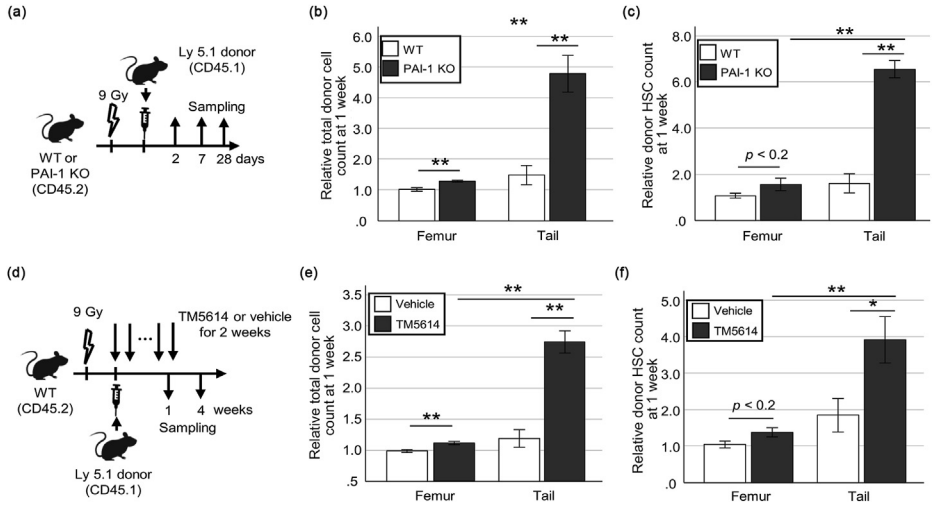

Fig. 2. Plasminogen activator inhibitor type-1 (PAI-1) knockout (KO) and specific PAI-1 inhibitor treatment facilitated hematopoietic regeneration especially in mouse tail vertebrae. (a): Experimental scheme of congenic transplantation using wild-type (WT) and PAI-1 KO mice. (b, c): Relative total donor (b) and donor hematopoietic stem cell (HSC) counts (c) in four leg bones were analyzed at 1 week. The values in WT mice were referred as a control. White and black bars represent WT and PAI-1 KO recipient mice, respectively (n = 6 for each group). (d): Experimental scheme of congenic transplantation in the presence of the PAI-1 inhibitor TM5614. (e, f): Relative total donor (e) and HSC counts (f) in four leg bone were analyzed at 1 week. The values in mice treated with vehicles were referred as a control. White and black bars represent vehicle- and TM5614-treated recipient mice, respectively (n = 6 for each group). *, $p < 0.05$; **, $p < 0.01$.

3.5. DIO mice exhibit higher PAI-1 concentrations and fibrinolytic system inhibition in the BM

We next sought to determine the role of PAI-1 in hematopoietic regeneration in BMA-rich mice. High-fat diet-fed mice (HFD mice, Fig. S3a) harbored abundant BMAs (Fig. 3a) and adipocyte-progenitor cells (Fig. S3b) and exhibited increased PAI-1 expression in the BM microenvironments (Fig. 3b, S3c).

To elucidate the effects of PAI-1 on the fibrinolytic system in HFD mice subjected to BM transplantation, we performed ELISA assays to quantify the amounts of PAI-1, tissue plasminogen activator (tPA), and plasmin in the BM fluid. Remarkably, we observed significantly higher levels of active PAI-1 in the BM of HFD mice relative to normal-fat diet-fed mice (NFD mice) at 1 week after transplantation (Fig. 3c), which was associated with lower concentrations of tPA, and plasmin (Fig. 3d and e). Furthermore, SCF in BM from HFD mice was lower than that from NFD mice (Fig. 3f). These results indicate that higher concentrations of PAI-1 inactivate the fibrinolytic system in BMA-rich DIO mice and thus decrease the production of SCF.

3.6. Hematopoietic regeneration is impaired in DIO mice

To clarify whether the higher PAI-1 concentration and inhibited fibrinolytic system in HFD mice would affect hematopoietic regeneration, we evaluated hematopoietic regeneration after treatment with 5-FU in HFD and NFD mice (Fig. 4a). Intriguingly, hematopoietic regeneration after 5-FU injection was significantly impaired in HFD mice relative to NFD mice (Fig. 4b). Next, to assess hematopoietic reconstitution in a BM transplantation model, lethally irradiated CD45.2 HFD or NFD mice were transplanted with CD45.1 congenic cells and euthanized 1 or 3 weeks later (Fig. 4c). Remarkably, both the total donor cell and HSC counts in the BM at 1 week were significantly lower in HFD mice than in NFD mice (Fig. 4d and e). In addition, the donor HSC cell counts at 3 weeks were still significantly lower in HFD mice than in NFD mice (Fig. 4e). These results indicate that hematopoietic regeneration after hematopoietic stress is impaired in DIO mice.

3.7. PAI-1 inhibitor treatment alleviated the impaired hematopoietic regeneration after cell transplantation in DIO mice

Finally, we sought to determine the effects of PAI-1 inhibition on hematopoietic regeneration in HFD mice. Initially, 5-FU-treated HFD mice were administered TM5614 or a vehicle control for 2 weeks consecutively (Fig. 4a). Notably, white blood cell counts in the peripheral blood of PAI-1 inhibitor-treated HFD mice were significantly higher than that of vehicle-treated HFD mice, although the counts did not reach the levels observed in the NFD mice (Fig. 4b).

Next, lethally irradiated CD45.2 HFD and NFD mice transplanted with CD45.1 congenic cells were treated with TM5614 or a vehicle control for 2 weeks consecutively (Fig. 4c). HFD mice treated with TM5614 exhibited significantly higher total donor cell counts in the BM when compared to their vehicle-treated counterparts; the counts in the former were comparable to those of vehicle-treated NFD mice (Fig. 4d). The donor HSC cell counts in HFD mice at 1 week were also significantly increased by TM5614 administration (Fig. 4e), and these effects were maintained even 3 weeks after cell transplantation (Fig. 4d and e). The increased hematopoietic regeneration in TM5614-treated HFD mice at 1 week was also confirmed by HE staining of the BM (Fig. S4a). Furthermore, PAI-1 inhibitor treatment significantly activated the fibrinolytic system in the BM of HFD mice, which was associated with higher levels of tPA and plasmin (Fig. 3d and e). Consequently, a higher concentration of SCF in the BM was observed in the TM5614-treated mice (Fig. 3f). Taken together, our results suggest that treatment with PAI-1 inhibitors may be a novel therapeutic approach that could alleviate fibrinolytic system impairment and facilitate hematopoietic regeneration after HSCT in DIO mice.

4. Discussion

In this study, we demonstrated the higher concentration of PAI-1 in BMA-rich microenvironments hindered hematopoietic regeneration by inhibiting the fibrinolytic system, and a blockade of PAI-1 activity facilitated hematopoietic regeneration in BMA-rich microenvironments after HSCT (Fig. S4b). This concept could be

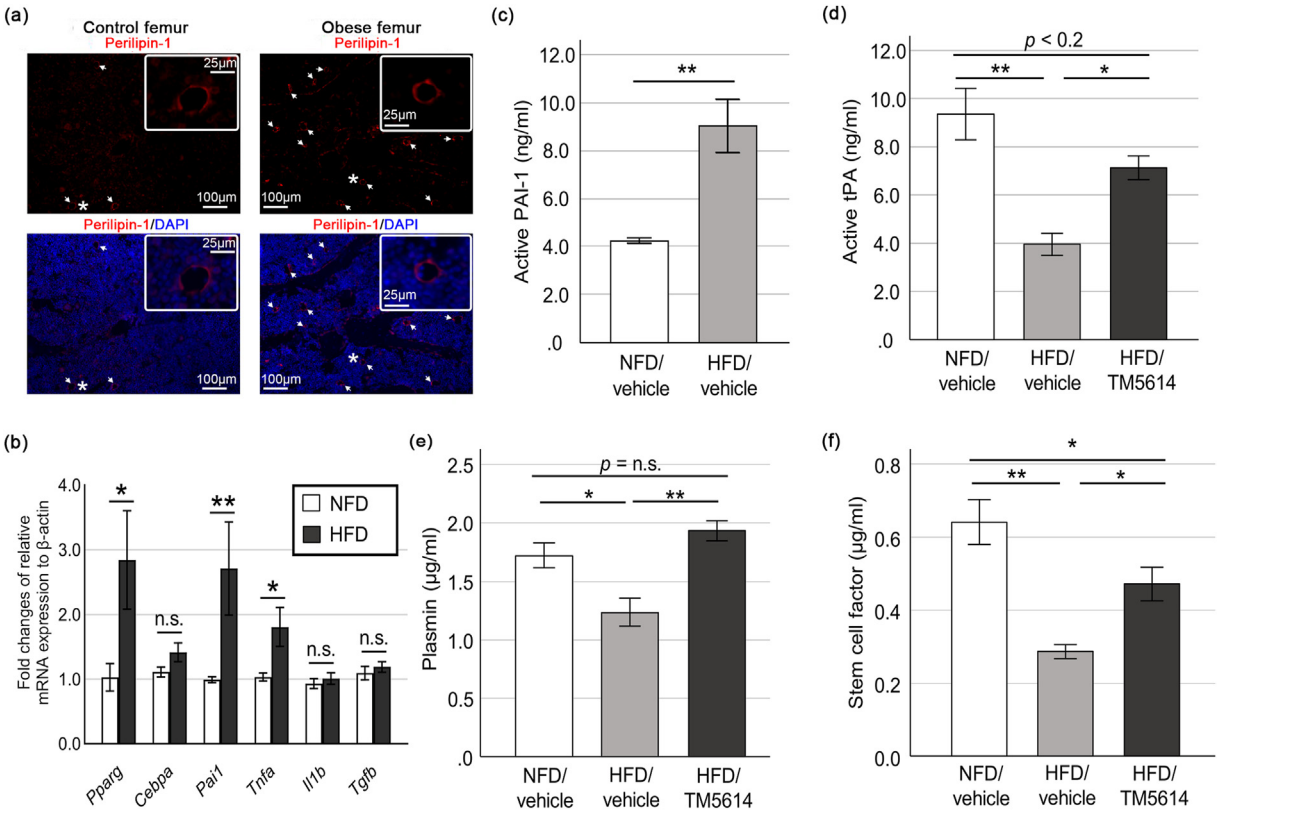

Fig. 3. The fibrinolytic system is suppressed by a higher concentration of plasminogen activator inhibitor type-1 (PAI-1) in the adipocyte-rich bone marrow (BM) in diet-induced obese (DIO) mice. (a): Representative images of BM sections of femurs from DIO and normal-weight mice. BM sections were stained with anti-perillipin-1 (red) and DAPI (blue). Arrows indicate perilipin-1-positive BMAs. Inserts are the higher magnification of the perilipin-1-positive BMAs (asterisk). Bars represent 100 μm and 25 μm. (b): qRT-PCR analysis of the expression of genes encoding adipokines and adipogenic transcription factors in $CD45^{-}CD31^{-}Ter119^{-}$ BM microenvironment cells from DIO mice relative to the expression in normal-weight mice (n = 4 per group). (c) The concentrations of active PAI-1 in the BM fluid were determined by ELISA (n = 4 in normal-weight and 5 in DIO mice). (d–f): The concentrations of active tissue plasminogen activator (d), plasmin (e), and stem cell factor (f) in the BM fluid were determined by ELISA (n = 6 for each group). *, $p < 0.05$; **, $p < 0.01$.

applied to human clinical studies of obese patients; however, other potential mechanisms may underlie the enhancing effect of PAI-1 inhibitors on hematopoietic recovery in BMA-rich microenvironments. First, PAI-1 inhibitors might affect intracellular PAI-1 activity in HSPCs. We previously showed that an intracellular PAI-1 blockade promoted HSPC motility after furin-dependent MT1-MMP maturation [21], which would affect the proliferation of HSPCs in PAI-1 inhibitor-treated obese mice. Second, a PAI-1 blockade might affect adipogenesis in the recipient mice. PAI-1 expression is associated with mitochondrial dysfunction in adipose tissues and contributes to adipogenesis via a positive feedback loop [22]. Thus, PAI-1 blockade might suppress adipogenesis in the BM after HSCT and indirectly enhance hematopoietic regeneration. In fact, the facilitated hematopoietic regeneration by the inhibition of adipogenesis has been reported in several studies [10–12]. A decrease in adipokines other than PAI-1, including neuropilin-1 [23], or the adipogenic suppression itself might have affected the results.

Once the fibrinolytic system is activated, metalloproteinase-mediated SCF secretion is a key factor in enhanced HSPC proliferation [15,16]. Here, we demonstrated the association between PAI-1 production in BMAs and a decreased SCF concentration in the BM of obese mice. However, distinct subtypes of BMAs with different characteristics have been identified (constitutive and regulated BMAs) [24], and gene expression analyses revealed that BMAs express a wide variety of hematopoietic and inflammatory cytokines [25]. Emerging evidence suggests that BMAs also facilitate hematopoietic regeneration directly by secreting SCF [26] and adiponectin [27]. Whether BMAs increase or decrease the SCF concentration in the BM remains paradoxical. BMAs might directly produce SCF during the early period after HSCT [26] and would inhibit external SCF secretion from BMSCs. Hematopoietic recovery after HSCT increases in response to suppressed adipogenesis [10–12]. Therefore, BMAs should exert both positive and negative effects on hematopoiesis. Further studies are needed to better understand the biological characteristics of BMAs and how these cells regulate hematopoietic regeneration from the perspective of both intrinsic and extrinsic factors.

The HSPC-supportive role of BMAs within the functional BM microenvironment has recently been recognized [9]. We demonstrated an association of PAI-1 in the BM with suppressed hematopoietic regeneration; however, this association does not necessarily mean that PAI-1 is associated with the exhaustion or loss of HSC functions; rather, PAI-1 might contribute to the maintenance and reservation of the HSC function by forcing HSCs to remain quiescent. In fact, the frequency and functions of long-term HSCs was maintained in the BMA-rich tail vertebrae [10]. Furthermore, BMAs secrete TGF-β [23] and CXCL12 [25], which are associated with HSC retention and maintenance. Obese individuals exhibit BMA-rich microenvironments even in the steady state [4]. Possibly, these abundant BMAs prevent HSCs from exhaustion via the PAI-1-mediated suppression of hematopoietic proliferation. Previous studies have shown that inflammatory cytokines, IL-1β, induce granulopoiesis in the BM of obese subjects [6], and the resulting chronic inflammatory stress can lead to HSC exhaustion

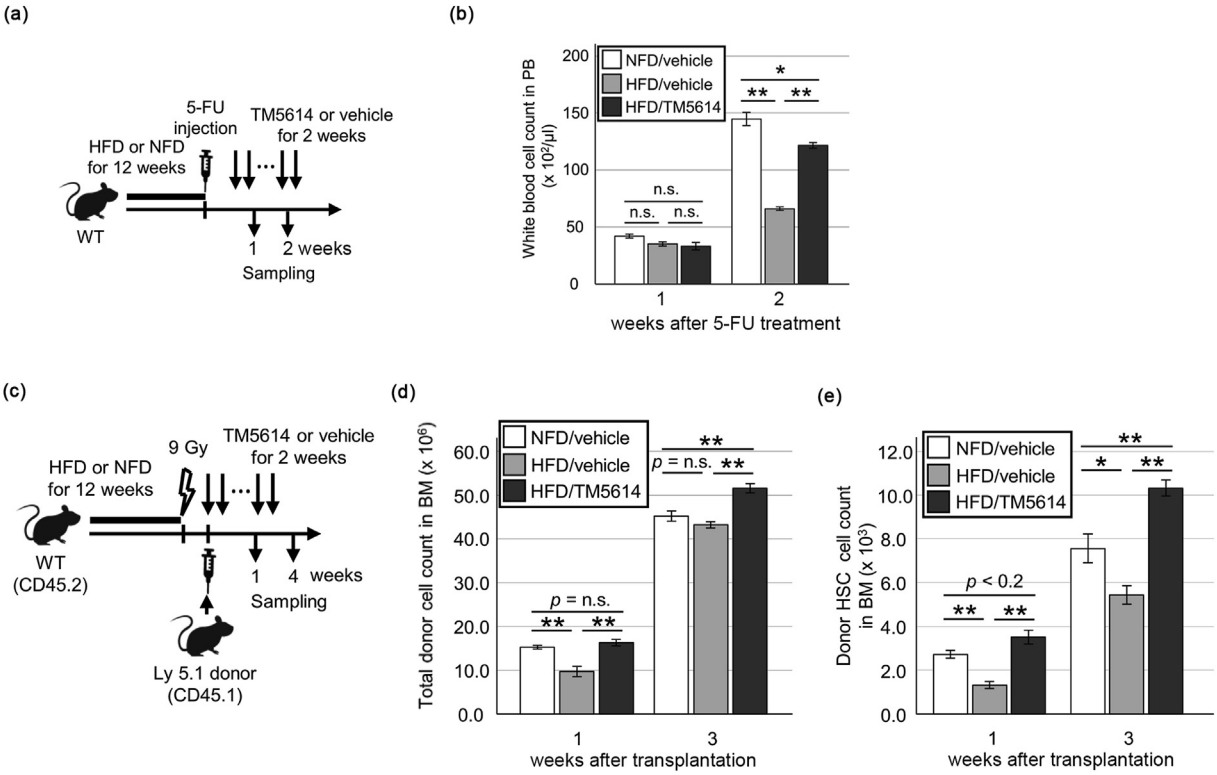

Fig. 4. Impaired hematopoietic regeneration in diet-induced obese (DIO) mice was alleviated by the administration of a plasminogen activator inhibitor type-1 (PAI-1) inhibitor. (a): Experimental scheme of hematopoietic regeneration after treatment with 5-fluorouracil (FU, 150 mg/kg). (b) White blood cell counts in the peripheral blood after 5-FU treatment. White, gray, and black bars represent normal-weight mice treated with vehicles and DIO mice treated with vehicles or TM5614, respectively (n = 3–4). (c): Experimental scheme of congenic transplantation using DIO and normal-weight mice. (d, e): Total donor (d) and hematopoietic stem cell counts (e) in the four leg bones were analyzed at 1 and 3 weeks after transplantation. White, gray, and black bars represent normal-weight mice treated with vehicles and DIO mice treated with vehicles or TM5614, respectively (n = 6 per group). *, $p < 0.05$; **, $p < 0.01$.

[28]. Notably, previous DIO mouse model studies have demonstrated the loss of long-term HSCs or hematopoietic disruption [3,5]. A similar principle can be observed in the BM of older individuals, in which BMAs constitute a majority of the microenvironment [6].

In conclusion, our results indicate that PAI-1 hinders hematopoietic regeneration in BMA-rich microenvironments. Moreover, a blockade of PAI-1 activity can alleviate the impaired hematopoietic recovery in a BMA-rich microenvironment. Pharmacological PAI-1 blockade may be a novel therapeutic approach to increasing hematopoietic regeneration after HSCT in BMA-rich patients.

Declaration of competing interest

The PAI-1 inhibitor used in this study is protected as intellectual property. T.M, K.A., and T.Y are listed as inventors in the patent application.

Acknowledgments

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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.2021.04.017.

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