Contribution of ADAMTS13 to the better cell engraftment efficacy in mouse model of bone marrow transplantation

The adhesive protein von Willebrand factor (VWF) plays an essential role in physiological hemostasis, mediating platelet adhesion and aggregation under high shear stress conditions.[1,2] The VWF-cleaving protease ADAMTS13 precisely down-regulates VWF activity to avoid pathological intravascular thrombosis in the microvasculature, including arterial capillaries, where blood flow typically creates high shear stress.[1-3] Indeed, the functional deficiency of ADAMTS13 is known to cause thrombotic thrombocytopenic purpura, a typical thrombotic occlusion of the microvasculature.[2,4,5] Thus, the proper equilibrium between VWF and ADAMTS13 is necessary for robust microcirculation *in vivo*. In this context, we hypothesized that ADAMTS13 might contribute to better donor cell homing and engraftment in various cell therapy approaches, in which fluent blood flow in the microcirculation system could be critical. To test this hypothesis, we investigated the role of ADAMTS13 on donor cell engraftment using a bone marrow transplantation (BMT) model in ADAMTS13 gene-deleted (*Adamts13* -/-) mice.

Adamts13 -/- (KO) mice were back-crossed for more than 15 generations to the C57BL/6 background, as described.[6] Wild-type (WT) mice (C57BL/6-background) were purchased from Japan SLC (Shizuoka, Japan). All mice used in this study were 8-12 weeks old with body weights of 25-30 grams. Mouse experiments were performed in accordance with protocols approved by the Ethics Review Committee for Animal Experimentation of Nara Medical University. In the BMT experiment, recipient WT or KO mice were conditioned for cellular transplantation with lethal total body irradiation (TBI: 5.5 × 2 = total 11 Gray) using a cesium irradiator (MBR-1520, Hitachi, Tokyo,

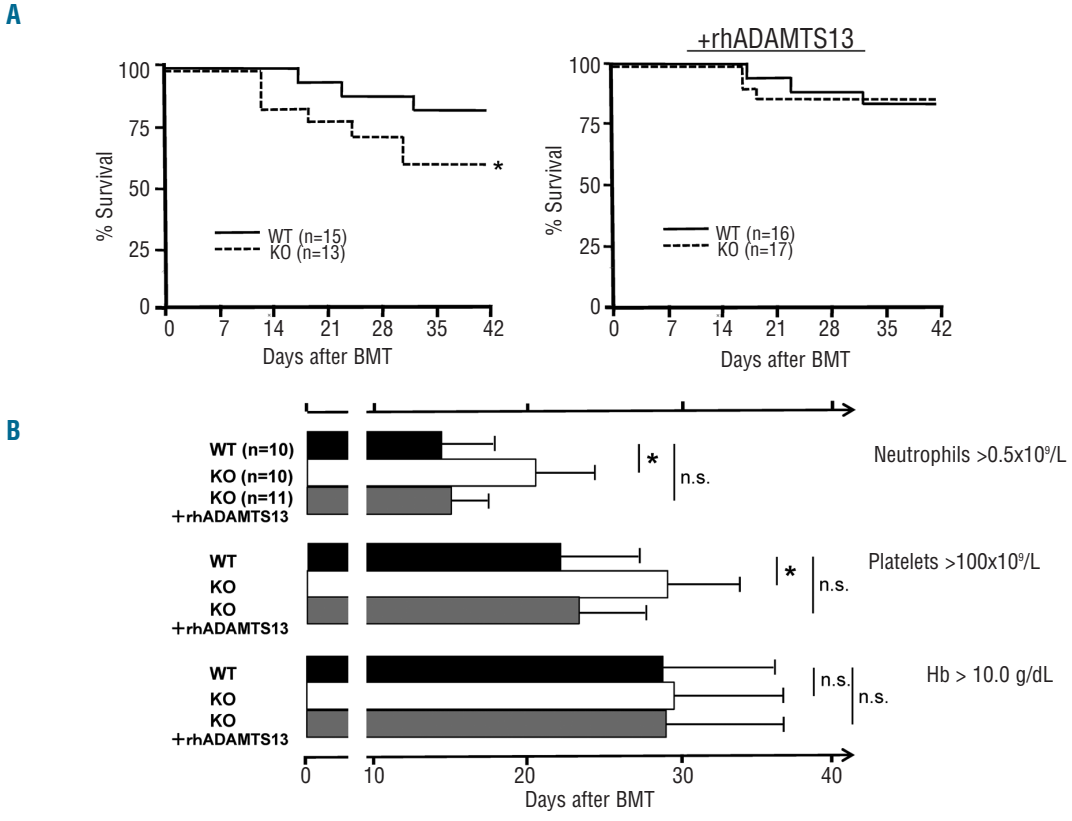

Figure 1. Survival rates and bone marrow suppression of wild-type (WT) or *Adamts13* $^{-/-}$ (KO) mice receiving TBI and subsequent BMT. (A) Kaplan-Meier analysis of survival rates of WT or KO mice receiving TBI and BMT. GFP-positive donor bone marrow cells (5 × 10^6/mouse) were transplanted to sex-matched WT (n=15) or KO (n=13) mice within 6 hours after TBI (total dose of 11 Gray/mouse) via tail vein. In some indicated experiments (right panel), recombinant human ADAMTS13 (rhADAMTS13; 5 μg/mouse, equivalent to 200 U/kg) was added to the donor bone marrow cell suspension prior to cellular transplantation. Significance of survival studies was quantified using Kaplan-Meier analysis and log rank tests. Note that the survival rate of KO mice began declining significantly (*P<0.05) at Day 14 of BMT, as compared to the WT mice (left panel; WT: 100% *vs.* KO: 76.9%). Following bolus administration of rhADAMTS13, this impaired survival rate in KO mice (WT: 81.0% *vs.* KO: 61.5% at Day 35) improved and became nearly indistinguishable from WT mice (see right panel). (B) Sequential peripheral blood analysis of WT or KO mice after TBI and BMT. Recipient mice were anesthetized using isoflurane inhalation, and 70 μL of blood was collected from the saphenous vein. Complete blood counts of recipient WT (n=10) or KO (n=10) were determined with an automatic blood cell counter (pocH®-100i: Sysmex, Kobe, Japan) every three days following BMT. Each bar represents the mean ± standard deviation (SD) duration that neutrophils counts were > 0.5x10^3/L, platelet tcounts were > 100x10^3/L, or hemoglobin values (Hb) were > 10.0 g/dL. Differences between groups were evaluated by Student's t-test. Note that nadir periods of KO mice are significantly (*P<0.05) longer than those of WT mice with regard to neutrophil and platelet counts (WT: 14.4 ± 3.3 and 22.4 ± 3.5 days *vs.* KO: 20.2±3.8 and 28.5±4.8 days, respectively), while no differences between these 2 groups are seen in Hb (WT: 28.2 ± 7.8 days *vs.* KO: 29.7 ± 7.5 days). These nadir period prolongations were improved by rhADAMTS13 (n=11) to an extent comparable to those of WT (n.s.: not significant).

Japan). Bone marrow cells to be transplanted were collected from femurs and tibias of donor green fluorescence protein (GFP) mice[7] (purchased from Japan SLC: C57BL/6-background), as described.[8] After removing the red blood cells by lysing with Tris-buffered ammonium chloride, suspended donor bone marrow mononuclear cells were transplanted into irradiated sex-matched recipient mice via tail vein. In some indicated experiments, recombinant human ADAMTS13 (rhADAMTS13), which was prepared as previously described,[9] was added to the donor bone marrow cell suspension prior to cellular transplantation. The VWF-cleaving activity of rhADAMTS13 was determined by *in vitro* FRETS-VWF73 assay.[10]

Kaplan-Meier analysis showed that the mean survival rate of KO mice receiving TBI and subsequent BMT was significantly lower than that of WT mice starting at Day 14 after BMT, and recombinant ADAMTS13 restored the survival rate of KO mice to that of WT mice (Figure 1A). Since all WT and KO mice that underwent TBI without BMT died within 21 days (*results not shown*), the mortality rates under our experimental conditions most likely depended upon the cell engraftment efficacy during BMT and indicate an important contribution of ADAMTS13 in this regard. Indeed, peripheral blood analysis following BMT revealed the longer nadir period in KO mice with regard to neutrophils and platelets (Figure 1B), which was shortened significantly by recombinant ADAMTS13, with the resulting nadir periods comparable to those of WT mice (Figure 1B).

In addition to the above long-term observation experiment, some recipient mice were sacrificed at Days 1, 7, and 14 after BMT to check the extent of donor cell engraftment to the bone marrow and to assess the pathohistological conditions of major organs. After removing the red blood cells, the recipients' bone marrow was collected from the femurs and tibias and used to assess donor cell engraftment efficacy based on the percentage of GFP-positive cells rela-

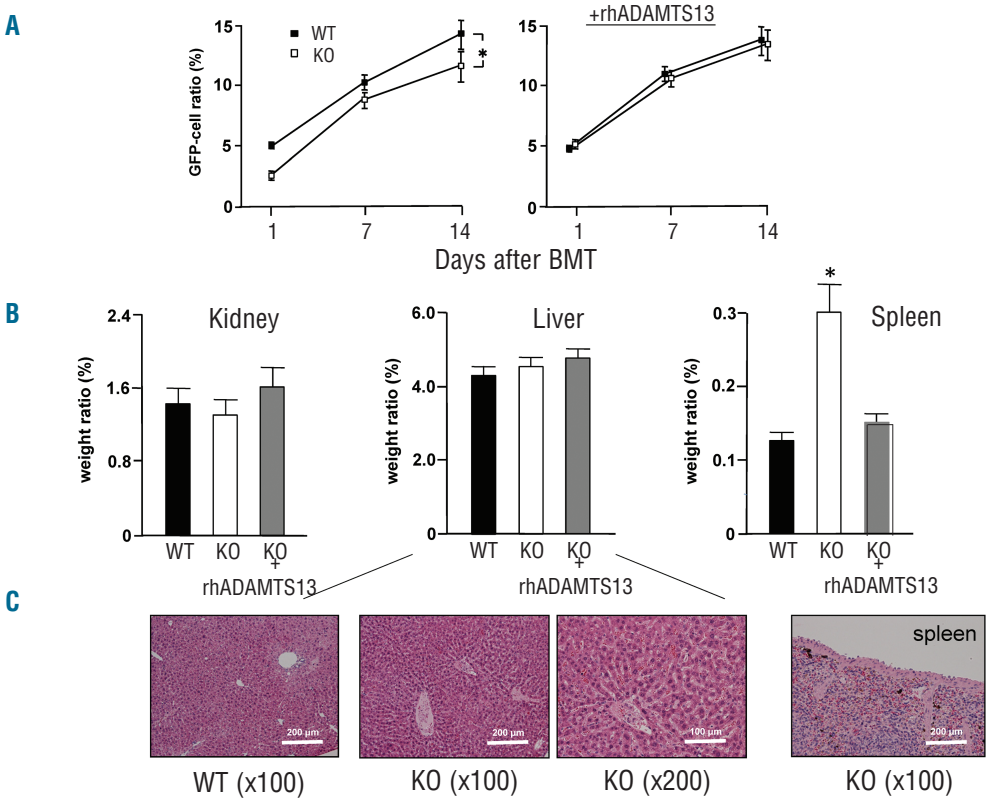

Figure 2. Bone marrow analysis and pathohistological studies in WT or KO mice that received TBI and subsequent BMT. These series of experiments, in which the recipient mice were sacrificed at Days 1, 7, and 14 after BMT (n=5 each), were performed independently of the long-term observation experiments in Figure 1. (A) Flow cytometric analysis of bone marrow cells from WT or KO mice that received TBI and BMT. Each data point represents the average ± SD of "GFP-cell ratio," the percentage of GFP-positive cells relative to total mononuclear cells in bone marrow. Note that a significant (*$P < 0.05$) reduction of donor GFP-positive cells in KO mice is already seen at Day 1 and continues throughout the observation period. In terms of cell propagation in KO mouse marrow, GFP-cells gradually increased in a time-dependent manner similar to that of WT (left panel). This GFP-cell reduction in KO mice was eliminated by rhADAMTS13 (see right panel). (B) Macroscopic findings of major organs in mice sacrificed at Day 7. Each bar represents the average ± SD of "weight ratio," the percentage of each organ weight (kidney, liver, or spleen) relative to total mouse body weight. In terms of macroscopic appearance, no particular differences were seen between WT and KO mice, except for a larger spleen in KO mice (*results not shown*). In fact, the calculated weight ratio confirmed the significant (*$P < 0.05$) splenomegaly in KO mice, which was eliminated by rhADAMTS13 administration. Mild splenomegaly, the extent of which was improved, remained in the corresponding Day 14 samples of KO mice (*results not shown*). (C) Microscopic findings of liver or spleen in mice sacrificed at Day 7. Images displayed are representative of 5 independent mouse samples. The liver samples with hematoxylin-eosin staining (×100 or ×200: original magnification) demonstrate slight dilation of the portal and central veins as well as mild sinusoidal congestion in both WT and KO mice, albeit less pronounced in WT mice. KO mouse livers do not exhibit either typical thrombotic legions in micro-vessels or SOS-lesions. As consistent with macroscopic splenomegaly, mild congestion and external capsule hypertrophy are observed in spleen of KO mice. These microscopic findings are basically similar to the corresponding Day 14 samples (*results not shown*).

tive to total mononuclear cells using flow cytometer (BD LSR-II: Nippon Becton Dickinson Company Ltd., Tokyo, Japan). Consistent with the findings in the peripheral blood, flow cytometric analysis of recipient bone marrow revealed the reduction of donor GFP-positive cells in KO mice that was already significant at Day 1 after BMT (Figure 2A). The population of GFP-positive cells in the bone marrow of KO mice expands gradually in a time-dependent manner similar to that of WT mice (Figure 2A), suggesting that ADAMTS13 is likely to play a role in the initial donor cell homing rather than cell propagation in the bone marrow cell engraftment. Thus, our results could verify the initial hypothesis that ADAMTS13 may contribute to better donor cell homing to the target recipient marrow, a process that requires fluent blood flow in the microvasculature including arterial capillaries.

Thrombotic microangiopathy (TMA) is a well-recognized serious complication of BMT, especially in the liver in the form of sinusoidal obstruction syndrome (SOS), and is known to be associated with functional ADAMTS13 deficiency.[11] Our histological studies, however, have only confirmed mild congestion and sinusoidal dilatation in the liver as well as significant splenic enlargement and congestion in KO mice, without typical thrombotic or SOS lesions of microvessels (Figure 2B and C). These histological findings may be consistent with possible portal hypertension, perhaps reflecting transient occlusion of the microvasculature by enhanced leukocyte plugging or platelet micro-aggregate formation that may occur in systemic microcirculation. Thus, the reduced local microcirculation could result in the poor donor cell homing to bone marrow that was observed in KO mice. Indeed, some clinical symptoms of TMA with functional deficiency of ADAMTS13 are known to be labile and variable,[5] suggesting the existence of transient microvasculature occlusion that cannot be reproducibly demonstrated in final tissue sample sections.

Recent mouse model studies by us and others demonstrated that proper functional regulation of VWF by ADAMTS13 significantly ameliorates the severity of fatal arterial thrombosis in conditions such as cerebrovascular accident or myocardial infarction.[12-15] ADAMTS13 reduces VWF-dependent platelet microaggregate formation as well as inflammatory responses such as leukocyte accumulation at ischemic sites, both of which may result in local microvasculature occlusion.[5] Thus, this property of ADAMTS13 can protect against impaired microcirculation *in vivo*, and may also contribute to better donor cell homing and engraftment in various cell therapy approaches that require fluent blood flow in the microvasculature.

In conclusion, our results illustrate that the regulation of VWF-mediated thrombotic or inflammatory responses by ADAMTS13 may contribute to the improved systemic microcirculation critical for efficient donor cell homing and engraftment in BMT, suggesting a clinical therapeutic potential of ADAMTS13 in cell therapy approaches.

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