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CD133$^+$ bone marrow stem cells (BMSC) control platelet activation – Role of ectoNTPDase-1 (CD39)

Constanze Duhme[a,1], Nadja Lehwald[a,1], Beate E. Kehrel[b], Ellen Bauchrowitz[a], Arlette Ngepi[a], Moritz Schmelzle[c], Theodoros Kolokotronis[d], Tahar Benhidjeb[d], Martin Krüger[e], Kerstin Jurk[b,f], Wolfram T. Knoefel[a], Simon C. Robson[g], Jan Schulte am Esch[d,*]

[a] Department of Surgery A, University Hospital Duesseldorf, Germany
[b] Department of Anesthesiology Intensive Care and Pain Medicine, Experimental and Clinical Hemostasis, University of Muenster, Muenster, Germany
[c] Department of Surgery, Charite, Berlin, Germany
[d] Center of Visceral Medicine, Department of General and Visceral Surgery, Protestant Hospital of Bethel Foundation, Bielefeld, Germany
[e] Center of Visceral Medicine, Department of Gastroenterology and Internal Medicine, Protestant Hospital of Bethel Foundation, Bielefeld, Germany
[f] Center for Thrombosis and Hemostasis, Johannes Gutenberg-University, Mainz, Germany
[g] The Transplant Institute and Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard University, Boston, MA, USA

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ABSTRACT

Background: We previously demonstrated CD133+ bone marrow stem cells (BMSC) to promote hepatic proliferation for liver regeneration. Here, we evaluated the capacity of CD133+BMSC to utilize platelets for homing to vasculature and concomitant controlling their aggregability upon ADP stimulation.

Methods: CD133+BMSC and platelets were co-cultured along micro endothelial cells under variable flow conditions and tested for homing levels along vasculature. Aggregometry and FACS analysis were utilized to evaluate platelet reactivity following co-incubation ± CD133+BMSC. RT-PCR and FACS analyses served to characterize ADP degrading ectonucleoside triphosphate diphosphohydrolase-1 (ectoNTPDase-1/CD39) expression on various cell types.

Results: Platelets attracted human CD133+BMSC to autologous micro endothelium under shear stress unaffected by ADP stimulation. However, CD133+BMSC inhibited ADP-mediated platelet activation and aggregation. Latter was dependent on ectoNTPDase-1 expression levels. Platelet aggregatory control was increased with CD133+BMSC compared to CD133+PHSC. Different effects of those stem cell subtypes positively correlated with their FACS-detected expression levels of ectoNTPDase-1.

Conclusion: We provide evidence that CD133+BMSC are capable of controlling ADP-dependent platelet aggregation and activation by direct interaction dependent on cellular expression of ectoNTPDase-1. Whether different capacities of BMSC modulate platelet-depending thrombogenicity at sites of regeneration impact effectiveness and adverse event profiles of regenerative treatment requires further evaluation.

1. Introduction

We previously demonstrated CD133+ bone marrow stem cells (BMSC) to trigger acceleration of hepatic proliferation in mice [1] as well as in a clinical scenario of liver regeneration [2–5]. We demonstrated pronounced mobilization of CD133+BMSC in patients

Abbreviations: ADP, adenosine diphosphate; BMSC, bone marrow stem cell; CD39, see ectoNTPDase-1; dMEC, dermal microvasculature endothelial cells; EC, endothelial cells; EPC, endothelial progenitor cells; ectoNTPDase-1, ectonucleoside triphosphate diphosphohydrolase-1 (CD39); FACS, fluorescence activated cell sorting; G-CSF, granulocyte colony stimulating factor; GFP, green fluorescent protein; h, hour; HMEC-1, Human dermal microvascular endothelial cells; HSC, hematopoietic stem cells; HUVEC, human umbilical vein endothelial cells; LTA, Bohr-light-transmission aggregometry; MACS, magnetic activated cell sorting; min, minutes; mRNA, messenger RNA; PPP, platelet poor plasma; PRP, platelet rich plasma; RNA, ribonucleic acid; qRT-PCR, revers-transcriptase PCR; PHSC, peripheral hematopoietic stem cells

* Corresponding author at: Department of General and Visceral Surgery, Center of Visceral Medicine, Protestant Hospital of Bethel Foundation, Schildescher Str. 99, 33611 Bielefeld, Germany.
E-mail address: jan.schulteamEsch@evkb.de (J. Schulte am Esch).
[1] Contributed equally.

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following large liver resections compared to minor resections, latter to be mediated by hepatocyte growth factor (HGF) and stroma-derived factor-1 (SDF-1) [6]. Furthermore, we and others observed a major role for platelets in hepatic injury [7–9]. Increasing body of evidence points to a substantial role of platelets for various preclinical and clinical scenarios of hepatic regeneration following injury and resection, respectively [10–17]. Accumulating evidence points to platelets mediating stem and progenitor cell homing along the vascular interface in the scenario of regeneration [18–20]. We observed platelets to promote homing of CD133 + BMSC along various forms and species of endothelial cells (EC) in a p-Selectin/PSGL-1 manner. Further, we demonstrated platelets to enhance transmigration of CD133 + BMSC in the microenvironment of the warm ischemic damaged liver (data submitted for publication).

Despite the fact, that platelets show pronounced capabilities of progenitor cells interaction upon ADP-induced activation [21], we observed the platelet effect of promoting CD133 + BMSC homing to EC being broadly independent of platelet pre-stimulation with ADP. Early reports described monocytic leucocytes to inhibit platelet aggregation upon co-incubation in vitro [22,23].

Here, we questioned, whether CD133 + BMSC previously demonstrated to play a major role for hepatic regeneration do control exuberant aggregatory responses of platelets through interaction and bear a protective effect concerning ADP-stimulated platelet aggregation. Possible mechanisms, such as ADP-degrading ectoNTPDase-1 (CD39) on these stem cells were tested.

2. Material and methods

2.1. Cells and cell isolation

Primary human CD133 + BMSC were purified from bone marrow aspirates from patients undergoing abdominal surgery following written informed consent with approval from the local Ethics Committee (Heinrich-Heine-Universität, Duesseldorf, Germany; local approval no. 2852 and no. 2853). For purification, magnetic activated cell sorting (Miltenyi Biotec, Germany) according to manufacturer's instructions was utilized. Peripheral hematopoietic stem cells (CD133 + PHSC) were accordingly prepared from buffy coats of stem cell donors following granulocyte colony stimulating factor (G-CSF) treatment and stem cell apheresis in the Institute of Transplantation Diagnostics and Cell Therapeutics (ITZ) of the University of Duesseldorf. Purity was assessed by FACS analysis of each preparation using a BD FACSCanto flow cytometry system (BD Bioscience, Heidelberg, Germany). The following antibodies were used: human CD133/2-PE (293C3, Miltenyi Biotec), human CD45-APC (APC anti-human CD45, BD), human CD39-FITC (FITC anti-human CD39, BD) and human CD 34-PE-Cy7 (PE-Cy7 mouse anti-human CD34, BD).

For co-cultivation experiments with thrombocytes, platelet rich plasma (PRP) and platelet poor plasma (PPP) was prepared from BMSC and PHSC donors by blood centrifugation.

Human dermal microvascular endothelial cells (HMEC-1) were kindly provided by the Department of Anesthesiology, Intensive Care and Pain Medicine, Experimental and Clinical Hemostasis, University of Muenster, Germany and cultivated in endothelial medium (PAA, # U15-002) with glutamine, penicillin/streptomycin and growth factors hydrocortisone (1 µg/ml) and EGF (10 ng/ml) at 37 °C and 5% CO_2.

Human dermal fibroblasts (COS7) were used as internal control cells for light transmission assays (Born) and semiquantitative RT-PCR experiments.

2.2. Co-culture live cell assay

Human endothelial cells (HMEC-1) were cultured in fibronectin-coated capillaries of a live cell imaging system (BIOFLUX 200, Fluxion). We established a live cell imaging model for human endothelial cells co-cultured under shear stress with human CD133$^+$BMSC and platelet rich or poor plasma as control prepared from respective BMSC donors under different test conditions before infusion to the BIOFLUX system. The BIOFLUX System contains microfluidic flow channels that connect two wells of a 14-well microtiter plate. Air pressure is applied to induce flow conditions in capillaries at physiological shear forces of 0,2 and 1 dyn/cm^2. All experiments were performed in duplicates testing treatment versus control (DMSO/H_2O) conditions parallel with the same preparation of BMSC for 1 h followed by quantification of adherent CD133 + BMSC. To test the effect of pre-stimulation of platelets for adhesion of CD133 + BMSC, PRP was pre-stimulated with 0,5 µM ADP monitored by aggregometry utilizing the Born light transmission aggregometry (LTA) method in a Lumi-Aggregometer (Chronolog). Latter prevented over-stimulation with clot formation. Co-culture of CD133 + BMSC with platelets along micro endothelial cells under variable flow conditions with and without ADP-incubation was tested for homing levels along vasculature.

2.3. Born Light Transmission Aggregometry (LTA) assays

We performed in vitro studies on ADP-mediated platelet activation using the light transmission (Born) aggregometry to evaluate inhibition of platelet aggregation by CD133 + BMSC. In these studies, we measured the ability of agonist ADP to induce in vitro platelet to platelet activation by platelet aggregation testing. We used the classically Born aggregometry, in which PRP is stirred in a cuvette of the Born Aggregometer at 37 °C. The cuvette is placed between a light course and a photocell. When an agonist is added, the platelets aggregate and absorb less light leading to a light transmission increase which is detected by the photocell. 5 µM ADP as agonist was added to PRP (control) or PRP co-incubated with CD133 + BMSC in different cell concentrations of 8×10^3, 15×10^3 and 30×10^3 CD133 + BMSC. CD133 + BMSC-mediated inhibition of ADP-induced aggregation was compared to fibroblasts (COS7) as additional control.

2.4. FACS-measured fluorescence-labeled fibrinogen binding and BMSC/platelet association

FACS-based quantification of fluorescence-labeled fibrinogen (Fib-FITC) binding to activated platelets was utilized to evaluate platelet reactivity following co-incubation ± CD133 + BMSC and CD133 + PHSC. Platelet activation was measured by FACS detection of fluorescence-labeled fibrinogen (Fib-FITC) binding to platelets ± autologous CD133 + BMSC/PHSC co-incubation following concentration-dependent ADP-stimulation of platelets in ADP activation series. Platelet aggregation induced by different ADP concentrations (0–0.5 µM ADP) was measured as linear median Fib-FITC FACS intensity using a BD FACSCanto flow cytometry system. Fibrinogen-FITC was kindly provided by Dr. Kerstin Jurk (Department of Anesthesiology Intensive Care and Pain Medicine, Experimental and Clinical Hemostasis, University of Muenster, Germany).

Before FACS quantification, 5×10^4 platelets were incubated with CD133 + BMSC (5×10^4 cells in 100 µl) or CD133 + PHSC (1×10^5 cells in 100 µl), 150 µg/ml Fib-FITC antibody and following monoclonal FACS antibodies: human CD133/2-PE (293C3, Miltenyi Biotec), human CD34-APC (APC anti-human CD34, BD) and the platelet specific marker human CD42a-PerCP (PerCP anti-human CD42a, BD) in HBSS buffer for 10 min at room temperature. After addition of 1 mM $CaCl_2$, cell suspensions were incubated with different ADP concentrations for 5 min at room temperature and fixed in 8% PFA.

In binding association studies, stained cell suspensions were furthermore analyzed for double positivity for CD133+ cells and the platelet specific marker CD42. The double positivity of a subgroup of CD133+ progenitor cells for CD42 demonstrated a direct association of CD133+ progenitor cells and platelets after ADP stimulation.

2.5. CD39 expression analysis by semiquantitative RT-PCR and FACS

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) and FACS analyses served to characterize ADP-degrading ecto-nucleoside triphosphate diphosphohydrolase-1 (ectoNTPDase-1/CD39) expression on mRNA and protein level on various cell types. Total RNAs were isolated using the RNeasy Mini Kit (Qiagen Sciences) and reverse-transcribed by Superscript II Reverse Transcriptase (Invitrogen). cDNA was subsequently applied in a PCR reaction over 40 cycles using a Hot-Start Taq Polymerase (Roche). Samples were removed after 25, 30, 35 and 40 cycles and analyzed on a 2% agarose gel. Intensity of DNA bands were compared to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences used are as follows: human *ENTPD1*, 5′-GCCAGCAGAAAAGGAGAATG (forward) and 5′-TGGGACCTTGGAA TCACTTC (reverse); *ENTPD2*, 5′-GCTCCTGTGATGTTCCAGGT (forward) and 5′-TCAAAGGGGTACTGGGTCAG (reverse); *ENTPD3*, 5′-ACC GGAGTGGTCAGTCAGTCAAAC (forward) and 5′-ATCTTCTCTCCTGCC ACGAA (reverse); *ENTPD8*, 5′-TCCACTTCCTGAACCTCACC (forward) and 5′-GCACCATGAACACCACTTTG (reverse); *CD133A*, 5′-CCTGGGG CTGCTGTTTATAT (forward) and 5′-GCAATCTCCCTGTTGGTGAT (reverse); and *GAPDH*, 5′-CACTGGCGTCTTCACCACCATGG (forward) and 5′-GGTTCACACCCATGACGAACATGG (reverse).

For FACS analysis, the previously indicated antibodies were used: human CD133/2-PE (293C3, Miltenyi Biotec), human CD45-APC (APC anti-human CD45, BD), human CD39-FITC (FITC anti-human CD39, BD) and human CD 34-PE-Cy7 (PE-Cy7 mouse anti-human CD34, BD). FACS detection was carried out on a BD FACSCanto flow cytometry system.

2.6. Statistics

Statistical analysis and graphing were performed using SMS Excel and SigmaPlot 14. All results are expressed as mean ± standard error (s.e.m.). Statistical significance was determined by Student's *t*-test, Welch test and ANOVA and significance was defined as $*p < 0.05$, $**p < 0.01$.

3. Results

3.1. Platelets attract human CD133 + BMSC to autologous micro endothelium under shear stress unaffected by ADP-stimulation

Since it was reported that platelets play a major role for hepatic injury and regeneration especially on liver sinusoidal endothelial cells (data submitted for publication) [8–12,24,25], we wanted to further investigate the modulating potential of platelets for the homing of CD133 + BMSC along vascular endothelium under flow conditions. To that respect, human micro vasculature endothelial cells (HMEC-1) were cultivated in capillaries of a life cell imaging system (BIOFLUX) (data submitted for publication) [26] in co-culture with platelet rich plasma (PRP) and human CD133 + BMSC under different shear stress conditions. Under shear stress of 1.0 dyn, platelets could significantly ($p < 0.005$) increase the number of adhering CD133 + BMSC to human micro-endothelium under flow conditions by a 4.3-fold increase (± 3.2; $p < 0.05$) when contrasted to platelet poor plasma (PPP) (Fig. 1A). However, under such shear force, pre-activation of platelets with ADP did not further augment the adhesion rate of CD133 + BMSC. If anything, ADP-stimulation rather abolished the platelet effect (1.7-fold ± 1.2, $p = 0.24$; Fig. 1A). Under reduced shear stress of 0.2 dyn, CD133 + BMSC adhesion along micro-vasculature appeared similar superior with PRP co-incubation when compared to PPP (2.6 fold ± 1.2; $p < 0.05$). ADP pre-stimulation of PRP demonstrated comparable levels of stem cell adhesion to EC (2.9 fold ± 1.0; $p < 0.01$).

3.2. CD133 + BMSC inhibit ADP-mediated platelets activation and aggregation in vitro

Next, we evaluated the platelet function regulatory impact of CD133 + BMSC subsequent to co-incubation of these cells. PRP was stimulated with ADP in presence of variable concentrations of CD133 + BMSC or in absence of such cells. Light Transmission (Born) aggregometry was utilized to quantify aggregatory responses. Stimulation of PRP with 5 μM ADP led to complete aggregation of platelets quantified as increase in light transmission. Co-incubation with CD133 + BMSC significantly reduced platelet aggregation upon ADP-stimulation in a dose-dependent manner (Fig. 1B). Adding 8×10^3 BMSC reduced ADP-mediated platelet aggregation by 50% when compared to a cell free control (27.8% ± 11.8 of max. aggregation vs. 60.9% ± 24.5, $p < 0.05$). This significant reduction was further augmented with increasing BMSC concentration resulting in a 69.2% reduction of control maximum aggregation with 30×10^3 BMSC ($p < 0.005$) (Fig. 1C).

A second assay, utilizing FITC-marked fibrinogen binding to activated platelet GPIIb/IIIa receptors, quantified with FACS analysis, was applied to confirm latter effect on inhibition of platelet function by CD133 + BMSC. Increasing ADP concentration positively correlated with fibrinogen binding to platelets (Fig. 1D). As a matter of fact, platelets demonstrated similarly to the Bohr-method significantly reduced activation-levels upon ADP-stimulation when co-incubated with CD133 + BMSC when contrasted to PRP without stem cell co-incubation (0.125 μM ADP: 32.7 ± 17.9 vs. 86.0 ± 34.3; 0.5 μM ADP: 117.0 ± 55.1 vs. 174.0 ± 67.8; $p < 0.05$; Fig. 1D).

3.3. The role of ectoNTPDase-1 for inhibition of ADP-dependent platelet activation by CD133 + BMSC

Interestingly, our data showed that modulation of ADP-induced platelet aggregation is a specific effect of CD133 + BMSC since control cells (fibroblasts; Cos7-cells) demonstrated no effect on platelets aggregation as shown by trans-luminescence assay (Fig. 2A). In order to further investigate the molecular mechanism of CD133 + BMSC for ADP-dependent platelets aggregation inhibition, we investigated CD39, known as ectoNTPDase-1, which hydrolyzes the strong platelet activator adenosine diphosphate (ADP) to adenosine monophosphate. We have previously shown that CD39 is highly expressed by a sub-set of murine and human HSCs and is critical for chemotaxis and recruitment of these cells from bone marrow to the liver [1]. CD133 + BMSC show a strong CD39 expression at mRNA level in contrast to control fibroblasts, which demonstrate almost no CD39 expression as shown by qRT-PCR (Fig. 2B).

3.4. Correlation of ectoNTPDase-1 expression on CD133 + BMSC and CD133 + PHSC with differential platelet function modulating effects of these BMSC-subtypes

CD133 + BMSC directly gathered from bone marrow aspirates and CD133 + PHSC prepared from apheresis-preparations from peripheral blood subsequent to reduce ADP-stimulated platelets activation quantified by FITC-marked fibrinogen binding to platelet GPIIb/IIIa. However, CD133 + BMSC demonstrated a significantly superior potential of controlling ADP-mediated platelet activation demonstrated as two-fold reduction of fibrinogen binding to platelets under ADP-stimulation if contrasted to CD133 + PHSC (63.1% ± 9.7% vs. 32.5% ± 14.7% reduction of aggregation responses as measured under stem cell free conditions all experiments subsequent to co-incubation with 0.125 mM ADP; Fig. 3A).

FACS analysis of events double positive for CD133 + /CD42 + revealed platelets to be significantly higher associated with CD133 + BMSC if contrasted to CD133 + PHCS under non-stimulated, ADP-free conditions (10.5 ± 2.1 vs. 6.5 ± 1.5, $p < 0.005$). However,

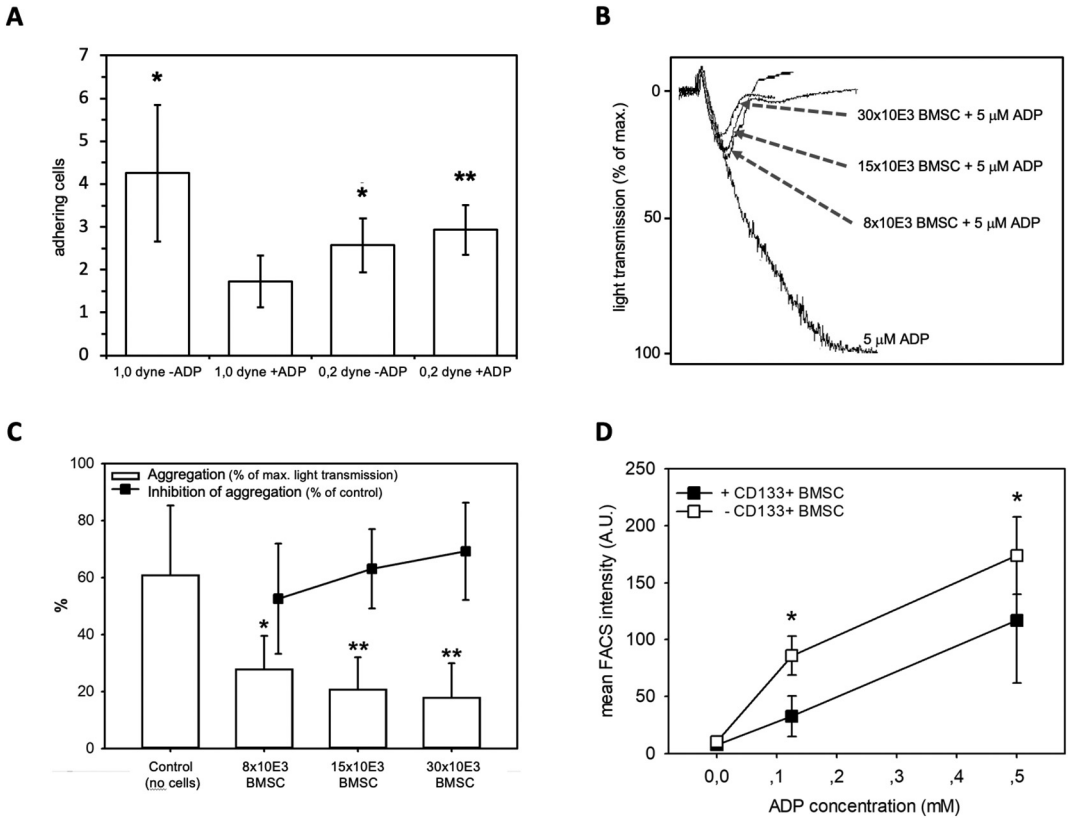

Fig. 1. Role of platelets for ADP-mediated platelets activation and aggregation under shear stress.
A. Increased CD133+ bone marrow stem cells (CD133+BMSC) adherence to HMEC-1 under shear stress (1 and 0.2 dyn) after platelet pre-incubation (PRP) ± ADP pre-stimulation (2 mM) in comparison to platelet poor plasma (PPP)-pre-incubation as control. B. ADP (5 µM)-induced platelet aggregation is inhibited by CD133+BMSC as measured by Light Transmission (Born) aggregometry. C. CD133+BMSC co-incubation results in a concentration-dependent inhibition of light transmission and platelet aggregation. Control: PRP + 5 µM ADP, no cells added. D. ADP-induced concentration-dependent fluorescence labeled fibrinogen binding to platelets as marker of activation is inhibited subsequent to co-incubation with CD133+BMSC as measured by FACS analysis. *$p < 0.05$; **$p < 0.01$; n.s. - not significant.

these differences were abolished by pre-stimulation of platelets up to 0.5 mM ADP (Fig. 3B).

Since we have already demonstrated a strong ectoNTPDase-1 expression (Fig. 2B) for CD133+BMSC, we further explored this potential molecular mechanism for differential thrombogenic control capacity of the two BM-subtypes tested here. qRT-PCR analyses revealed a strong CD39 yet comparable expression at a transcriptional level for both, CD133+BMSC and PHSC (Fig. 3C). However, protein levels quantified

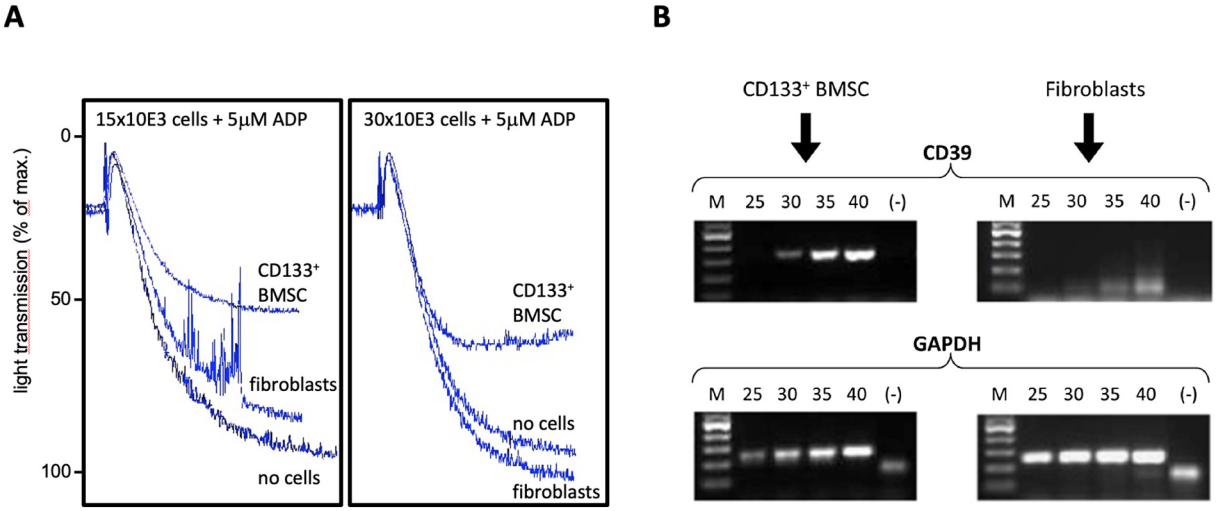

Fig. 2. Role of CD39 and CD133+BMSC for impaired ADP-dependent platelet activation.
A. Light Transmission Aggregometry demonstrated a specific effect of CD133+BMSC for ADP-induced platelet aggregation inhibition since control cells (fibroblasts) showed no effect for platelet aggregation (PRP + 5 µM ADP). B. Semi-quantitative RT-PCR analysis for CD39 expression revealed pronounced CD39 expression in CD133+BMSC compared to no CD39 expression in fibroblasts. GAPDH was used for internal housekeeping control. M: marker, (−): negative PCR control, 25, 30, 35, 40: PCR cycles.

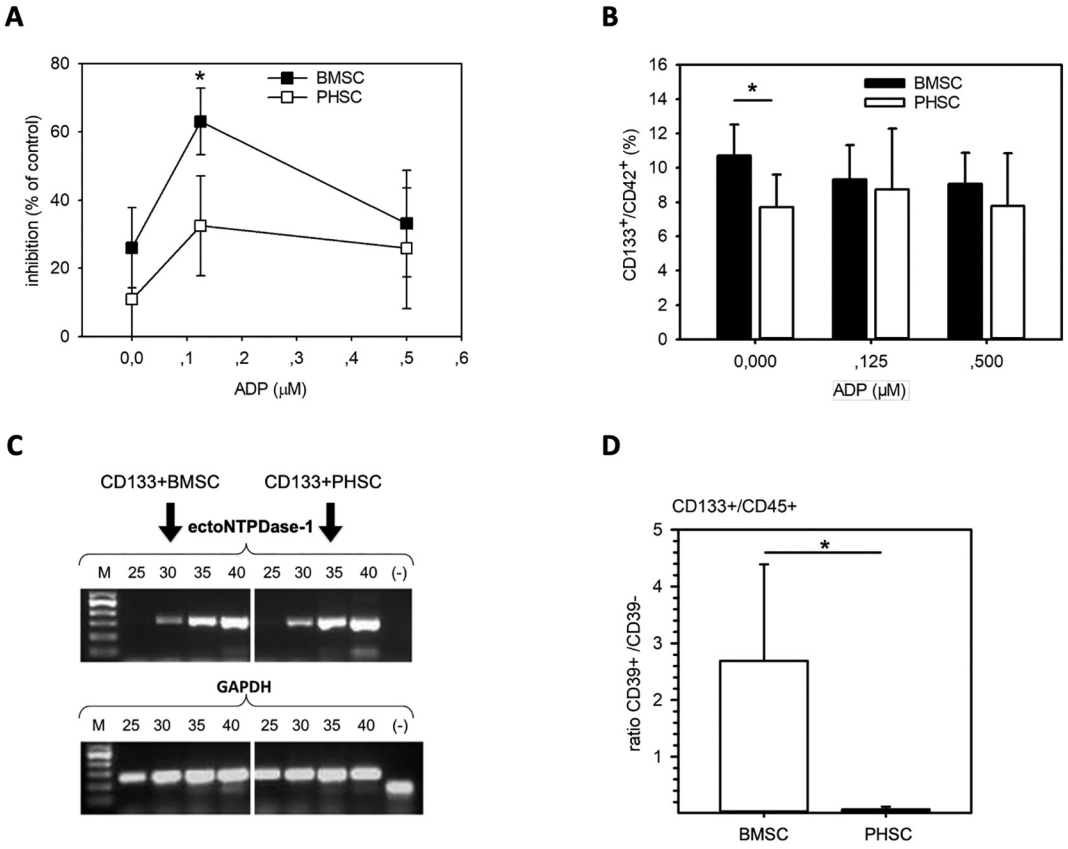

Fig. 3. Differential NTPDAase1 expression and platelet function modulating effect of bone marrow gathered in contrast to peripherally collected CD133+ hematopoietic stem cells.

A. Subsequent to G-SCF-treatment of donors, peripheral hematopoietic stem cells (PHSC) double positive for CD133 and CD45 are inferior in their ability to inhibit ADP-induced platelet aggregation if contrasted to BMSC as measured by FACS analysis. Control: PRP + 5 µM ADP, no cells added. B. The direct association of CD133+ progenitor cells with platelets is verified by FACS analyses. Differences in platelets co-associating with CD133+PHSC and CD133+BMSC respectively represented by the percentage of CD133+ cells double positive for platelet-specific CD42 are leveled following ADP-stimulation C. Comparable ectoNTPDase (CD39) expression on the mRNA-level in CD133+BMSC and CD133+PHSC as quantified by semi-quantitative RT-PCR analysis. GAPDH was used for internal housekeeping control. M: marker, (−): negative PCR control, 25, 30, 35, 40: PCR cycles. D. Substantially increased ectoNTPDase-1 (CD39) protein expression in CD133+BMSC compared to CD133+PHSC was measured by FACS analysis and is demonstrated as differential ratios of CD39+ and CD39− cell fractions among CD133/CD45 double-positive cells. $p < 0.05$; *$p < 0.01$; n.s. - not significant.

by FACS analysis showed ectoNTPDase-1 expression of CD133+BMSC to be substantially superior to CD133+PHSC. The ratio of CD39+ and CD39− cells in the CD133/CD45 double positive subpopulation was significantly higher in BMSC compared to PHSC (2.67 ± 3.4 vs. 0.07 ± 0.1; $p < 0.005$; Fig. 3D).

4. Discussion

In the present study, we provide evidence that CD133+BMSC are capable of controlling ADP-dependent platelet aggregation and activation upon direct interaction. This modulation of platelet function was dependent on cellular expression of ectoNTPDase-1 (CD39). The platelet function controlling capacity of CD133+BMSC was superior to CD133+PHSC, correlated with ectoNTPDase-1 expression but independent of direct association levels of stem cells with platelets.

Our data on platelets to attract human CD133+BMSC to micro endothelium under shear stress are in line with previous reports showing that endothelial progenitor cells (EPC) formed heterotypic aggregates with resting platelets and interact in vitro with platelets under static and flow conditions. This interaction may be a central mechanism for homing of such cells to vascular injury sites. The interaction of progenitor cells and platelets was greatly enhanced when platelets were activated subsequent to ADP-stimulation [27]. However, we showed that pre-activation of platelets with ADP did not further increase the adhesion rate of CD133+BMSC to EC, latter under variable shear-conditions.

Our hypothesis was that CD133+ BMSC utilize platelets as vehicle for improved homing along vasculature to the damaged liver while concurrently controlling platelet-mediated thrombogenicity. In fact, we demonstrated CD133+BMSC to inhibit ADP-mediated platelets activation and aggregation in vitro in two different assays in a dose-dependent manner. These data coincide with early reports describing monocytic leucocytes to inhibit platelet aggregation upon co-incubation in vitro [22,23]. Furthermore, aggregation of platelets co-incubated with monocytes rather than granulocytes resulted in impaired responsiveness of platelet aggregometry in trauma patients [28]. EPC for their part were similar shown to inhibit platelet aggregation and reduce thrombus formation latter via a cellular mechanism involving binding to platelet P-selectin [27].

Recently, we demonstrated EctoNTPDase-1 to improve CD133+ hematopoietic stem cell recruitment and to promote liver regeneration in mice and humans after partial hepatectomy [1]. In addition, we demonstrated ectoNTPDase-1 to control ADP-dependent platelet aggregation [29]. Here, we questioned whether ADP-degrading ectoNTPDase-1 (CD39) plays a role for CD133+BMSC to control ADP-dependent platelet activation mechanisms. In contrast to CD133+BMSC, fibroblasts (cos-7-cells) utilized here as control cells lacked to modulate ADP-dependent platelet aggregation similar to

previous observations [29]. We confirmed earlier findings of ectoNTPDase-1 to be absent in cos7-cells [29,30]. We demonstrated CD133 + BMSC to express ectoNTPDase-1 in keeping with data on its expression in mono and polymorph nuclear leucocytes (MNL and PMNL respectively). Adding such leukocytes (either MNLs or PMNLs) to platelets caused marked inhibition of ADP-induced aggregation [31]. Patients with increased white blood cell count demonstrated marked inhibition of ADP-induced platelet aggregation in correlation with raised levels of NTPDase-1 expression and activity if contrasted to individuals with normal white blood cell count [32,33].

Our finding of CD133 + PHSC prepared subsequent to G-CSF-stimulation to bear a significantly reduced potential to control platelet reactivity to ADP-stimulation if contrasted to CD133 + BMSC was in line with different anti-aggregatory effects of EPC of differential sources. Thus, subsets of EPC originating from peripheral blood MNC exhibited an anti-aggregatory effect on ADP-dependent platelets function inferior to the ones derived from CD34 + cord blood cells [34]. Although ectoNTPDase-1 expression of CD133 + PHSC was comparable to bone marrow derived CD133 + cells when evaluated on a transcriptional scale, characteristics on a translational level demonstrated ectoNTPDase to be markedly decreased on PHSC when compared to BMSC. We were able to show different platelet activation controlling potential among the two subtypes of BM-cells tested to be independent of differential degrees of direct interaction with platelets under ADP-stimulatory conditions.

We and others have observed differences of stem cells in their capacity to modulate platelet function depending on their source and mode of acquisition [34], which may have impact on effectiveness and spectrum of serious adverse events of protocols to treat liver damage, myocardial infarction or sepsis [35–37]. For bone marrow reconstitution subsequent to myeloablative treatment it was shown previously, that stem cell preparations from bone marrow aspirates vs. those from peripheral blood subsequent to donor G-CSF pre-treatment differ in reconstitutive and immunogenic characteristics [38]. Moreover, different effects of platelet aggregation control were observed for progenitor cell sub-populations originating from the same cell source but presenting with differential phenotypic characteristics [39].

In addition to our previous reports on EctoNTPDase-1 to improve CD133 + hematopoietic stem cell recruitment and to promote liver regeneration, the present study provides evidence for CD133 + BMSC to control ADP-induced platelet aggregation in an ectoNTPDase-1-dependent fashion. Whether diverse subsets of BM-derived CD133 + stem cells determined by source and mode of preparation and herewith associated with different capacities to modulate platelet depending thrombogenicity at sites of regeneration impact effectiveness and adverse event profiles in regenerative treatment protocols requires further evaluation.

Conflict of interest

The authors have nothing to disclose.

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Author contributions

Study conception, design and overall analysis and interpretation of data, drafting, revising the manuscript (C.D., N.L., J.S.a.E.), data acquisition (C.D., E.B., A.N., M.S), analysis and interpretation (S.C.R., C.D., N.L., K.J., B.E.K., J.S.a.E.), manuscript preparation (N.L., C.D., B.E.K., T.B., S.C.R., J.S.a.E.), conceptional contribution and manuscript revision (B.E.K., M.K., T.B., S.C.R., M.S., W.T.K., T.K.).

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