HHS Public Access

Author manuscript

Leukemia. Author manuscript; available in PMC 2023 May 08.

Published in final edited form as:

Leukemia. 2021 July ; 35(7): 1925–1932. doi:10.1038/s41375-020-01047-7.

DPP4+ exosomes in AML patients' plasma suppress proliferation of hematopoietic progenitor cells

Swathi Namburi[1], **Hal E. Broxmeyer**[2], **Chang-Sook Hong**[1], **Theresa L. Whiteside**[1], **Michael Boyiadzis**[1]

[1]University of Pittsburgh School of Medicine, UPMC Hillman Cancer Center, Pittsburgh, PA, USA

[2]Indiana University School of Medicine, Indianapolis, IN, USA

Abstract

Mechanisms by which acute myeloid leukemia (AML) interferes with normal hematopoiesis are under intense investigation. Emerging evidence suggests that exosomes produced by leukemia blasts suppress hematopoiesis. Exosomes isolated from AML patients' plasma at diagnosis significantly and dose-dependently suppressed colony formation of normal hematopoietic progenitor cells (HPC). Levels of HPC suppression mediated by exosomes of AML patients who achieved complete remission (CR) were significantly decreased compared to those observed at AML diagnosis. Exosomes from plasma of patients who had achieved CR but with incomplete cell count recovery (CRi) after chemotherapy suppressed in vitro colony formation as effectively as did exosomes obtained at AML diagnosis. Dipeptidylpeptidase4 (DPP4/CD26), a serine protease that cleaves select penultimate amino acids of various proteins, has been previously implicated in the regulation of hematopoiesis. DPP4 was carried by exosomes from AML plasma or leukemia cell lines. Leukemia exosomes which suppressed HSC colony formation had markedly higher DPP4 functional activity than that detected in the exosomes of normal donors. Pharmacological inhibition of DPP4 activity in AML exosomes reversed the effects of exosome-mediated myelosuppression. Reversing the negative effects of exosomes on AML hematopoiesis, and thus improving cell count recovery, might emerge as a new therapeutic approach to AML.

Introduction

In acute myeloid leukemia (AML) a state of profound bone marrow (BM) failure occurs regardless of a considerable variation in BM replacement by leukemic blasts [1]. The resulting cytopenias contribute significantly to high mortality and morbidity in AML patients. The mechanisms by which AML interferes with normal hematopoiesis are under intense investigation. It is hoped that these studies may lead to the development of new treatment modalities for AML. Leukemia invading the BM inhibits down-stream production of hematopoietic factors, impedes differentiation/maturation of normal hematopoietic progenitor cells (HPC), leads to microenvironmental BM remodeling, and alters the responsiveness of normal HSCs to cytokines [2–4]. We previously reported that AML

✉Michael Boyiadzis, boyiadzism@upmc.edu.

Conflict of interest The authors declare that they have no conflict of interest.

patients have significantly elevated plasma levels of exosomes [5, 6]. Exosomes are a subset of small extracellular vesicles (EVs) that range in size from 30 to 150 nm. They are produced by both normal and malignant cells [7, 8], and originate from the endocytic compartment of producer cells [9]. Exosomes have recently emerged as a universal intercellular communication system [10, 11]. The molecular/genetic cargo of exosomes reflects the content of their cells of origin, and this cargo residing in a protective protein/lipid bilayer is delivered to recipient cells without degradation. Because of their small size, exosomes freely circulate within the body and freely cross biological barriers [10, 11].

Exosomes in AML patients' plasma carry myeloid-blast markers and leukemia associated antigens (LAAs), and are enriched in immunosuppressive proteins [12]. Emerging data suggest that immunoinhibitory exosomes produced by leukemic blasts regulate normal hematopoiesis and simultaneously reprogram stromal and immune components of the BM, a new concept with potential clinical significance [13]. In vitro and in vivo studies in mouse models of leukemia demonstrated that exosomes released from AML cell lines and primary blasts suppress HPC functions indirectly through stromal reprogramming of the niche-retention factors and also as a consequence of AML exosome-directed miRNA delivery to HPCs [14–16]. However, components of the cargo that human AML exosomes use to interfere with normal hematopoiesis have not been investigated in detail.

Dipeptidylpeptidase 4 (DPP4/CD26) is a transmembrane glycoprotein with proteolytic activity that cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position [17, 18]. DPP4 has a widespread distribution: it is expressed in numerous tissues including intestinal and renal brush border membranes, by vascular endothelium, and by cells of the immune system. It participates in many physiological processes such as adhesion, migration, invasion, apoptosis, and immune modulation [19]. DPP4 plays a major role in glucose metabolism by N-terminal truncation and inactivation of the glucagon-like peptide-1 (GLP) and gastric inhibitory protein (GIP) [20]. DPP4 inhibitors have been used in clinical trials and were demonstrated to efficiently enhance the endogenous insulin secretion via prolongation of the half-life of GLP-1 and GIP in patients [21, 22]. DPP4/CD26 expression in hematological malignancies has been widely studied [23]. More recent reports have revealed that DPP4 regulates multiple aspects of hematopoiesis and is present as an active membrane-bound enzyme complex on AML blasts [24–26]. Using Western blots, we have identified that leukemia exosomes carry DPP4. However, it is not known whether DPP4 is on the exosome surface, whether it has enzymatic activity, or whether it negatively regulates human hematopoiesis. We hypothesized that $DPP4^{+}$ exosomes detected in the plasma of AML patients play a role in suppression of normal hematopoiesis and in hematologic recovery of AML patients following induction chemotherapy. We report here that exosomes from the plasma of AML patients at diagnosis are myelosuppressive and that exosomes in the plasma of patients who achieve complete remission with incomplete count recovery (CRi) inhibit normal human hematopoiesis in vitro. Furthermore, exosomes in AML patients at diagnosis carry DPP4 with high levels of enzymatic activity. Pharmacological inhibition of DPP4 activity in AML exosomes reversed the effects of exosome-mediated myelosuppression. This finding suggests that reversing the DPP4-mediated effects of exosomes on hematopoiesis could be considered as a new therapeutic approach to AML in the future.

Methods

Patients and normal donors

Venous blood was collected from AML patients at the time of diagnosis (n = 33) and following induction chemotherapy. Blood was also collected from normal donors (ND, n = 10). All patients and NDs signed informed consent forms approved by the University of Pittsburgh Institutional Review Board (IRB#960279). The methods used in this research were carried out in accordance with IRB guidelines and regulations.

Exosome isolation from plasma by mini size-exclusion chromatography

Exosomes were isolated from the plasma of AML patients and from supernatants of leukemia cell lines (Kasumi-1, ThP1, MLL-1) using mini size-exclusion chromatography (mini-SEC) on Sepharose 2B columns as previously described by us [12, 27]. Plasma was centrifuged at 2000 × g for 10 min at 4 °C and then at 10,000–14,000 × g for 30 min at 4 °C. Clarified plasma was passed through 0.22 μm-pore Millipore filter and used for exosome isolation by SEC performed using 1.5 cm × 12 cm columns (Bio-Rad, Hercules, CA, USA; Econo-Pac columns) packed with Sepharose 2B (Sigma-Aldrich, St. Louis, MO, USA). The column bed volume was 10 mL. Clarified plasma (0.5–1.0 mL) was loaded onto the column and eluted with PBS. 1 ml fractions were collected. Exosomes that eluted, in fraction #4 were harvested and their protein content, size, nanoparticle numbers, morphology, and molecular content were determined as previously described by us [12, 27]. The protein content in fraction #4 was determined using the Pierce BCA protein assay kit (Pierce Biotechnology, Rockford, IL), and the exosome protein concentration was expressed as μg protein/ml of precleared plasma loaded onto each mini-SEC column.

Western blots

In preparation for Western blots, exosome fraction #4 was concentrated by centrifugation on a 100 K Amicon Ultra 0.5 mL centrifugal filter (EMD Millipore, Billerica, MA, USA) at 5000 × g. Western blots were performed as previously described [12]. PVD membranes were incubated overnight at 4 °C with various antibodies (Abs) as indicated below. Exosomes (10 μg protein) were loaded in each lane and tested for the presence of exosome markers, including markers related to hematopoiesis, leukemia blasts markers, and Leukemia Associated Antigens (LAAs) as previously described [12]. The following Abs were used: anti-TGF-β1 (Cell Signaling, #3711, 1:1000); anti-TNF-a (Cell Signaling, 3707S, 1:1000); anti-TSG101 (Thermo Fisher, PA5–31260, 1:500); anti-EPOR (R&D, MAB307, 1:500); anti-CD26 (R&D, AF1180, 1:500); anti-CLL-1 (R&D, AF2946, 1:2000); anti-CD34 (Santa Cruz, sc-7045, 1:500); and anti-CD33 (Thermo Fisher, WM53, 1:500). Band intensities on exposed films were quantified using Image J software (NIH, USA). The examined band intensity was normalized to the intensity of TSG101 used as a marker of the exosome endocytic origin. The integrated pixel value was determined for each protein band by multiplying image intensity and band area after subtracting the mean background value.

Flow cytometry of exosomes captured on magnetic beads for DPP4 detection

Aliquots of exosomes (10 μg protein) isolated from AML patients' plasma, normal donors' plasma (ND), or the Kasumi-1 cell line were used for immunocapture. Exosomes were captured with streptavidin magnetic beads coated with biotinylated anti-CD63 mAbs (ExoCap™ Streptavidin Kit: MBL International Corporation, Woburn, MA, USA) for 12 h at 4 °C. After washing × 3 with PBS, exosomes on beads were stained with phycoerythrin conjugated anti-human DPP4 antibody (B45b) or isotype control (MOPC-173) (Biolegend, San Diego, CA, USA). On-bead flow cytometry was performed [28] using LSR For-tessa flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed with Kaluza 1.0 software (Beckman Coulter, Krefeld, Germany).

Colony forming cell assays

Human cord blood was obtained from the Institute of Transfusion Medicine, University of Pittsburgh, and mononuclear cells (MNCs) were isolated using Ficoll-gradient centrifugation. Exosomes isolated from AML patients' plasma or leukemia cell lines (1–50 μg protein) were used for the colony forming cell assays. Exosomes were suspended in PBS and plated in 35 mm culture dishes. MNCs were combined with methylcellulose media containing recombinant cytokines (SCF, 50 ng/ml; IL-3, 10 ng/ml; GM-CSF,10 ng/ml; and erythropoietin, 1 U/ml) for use with human cells (Stem Cell Technologies, Cambridge, Boston, MA, USA) as previously described [24]. Cells were plated in triplicate wells at the concentration of 5×10^4 per mL of media. The cell mixture was placed on top of the exosomes. Plates were incubated at 37 °C in 5% CO_2 for 12–14 d before colonies were enumerated using an inverted microscope. CFU-GM and CFU-GEMM colonies were differentiated and reported along with total colony counts. For reversal of inhibition experiments, exosomes were combined with either control media or Diprotin A (5 mM), a DPP4 inhibitor, for 4 h prior to plating [24, 26].

Dipeptylpeptidase-4(DPP4) activity assay

Aliquots of exosomes (10 μg protein) isolated from AML patients' plasma or leukemia cell lines were suspended in 50 μL PBS and plated in wells of a 96-well flat-bottom microtiter plate. The assay was carried out by combining the exosomes with 50 μL of the DPP4 substrate, Gly-Pro-aminoluciferin, and the buffer optimized for this assay (Promega, Madison, WI, USA). Plates were incubated at 37 °C for 60 min, and surface DPP4 enzyme activity was measured as luminescence signals using the Promega GloMax multidetection system. Diprotin A, a DPP4 inhibitor, was used for blocking DPP4 activity [24]. Titration experiments were performed using different doses of Diprotin A (0.1–0.6 mmol) to identify the optimal inhibitory dose of Diprotin A for the blocking experiments. Aliquots of exosomes (10 μg protein) were co-incubated with Diprotin A (0.2 mmol) for 30 min prior to the addition of DPP4.

Statistical analysis

Data were summarized by descriptive statistics (SPSS) using means and standard errors (SE). Statistical analysis was performed using GraphPad Prism. For parametric tests, unpaired *t* test or one-way ANOVA, and for non-parametric data, Mann–Whitney U or

Kruskal–Wallis tests were used. A p value of <0.05 was considered to be statistically significant.

Results

Exosomes from plasma of AML patients at diagnosis

Exosomes were isolated from plasma of 33 newly diagnosed AML patients prior to any therapy and from supernatants of leukemia cell lines (Kasumi-1, ThP1, ML2) using mini-SEC, as described above. Mean protein levels in exosomes isolated from the plasma of AML patients at diagnosis was 59 μg/mL (range, 26–155 μg/mL) (Fig. 1a). Transmission electron microscopy (TEM) of exosomes isolated from the AML patients showed the presence of vesicles ranging in size from 30 to 150 nm (Fig. 1b, c). Western blot analyses indicated that exosomes isolated from plasma of AML patients were $TSG101^+$ and carried DPP4, TGFβ-1, TNF-α and several LAAs (Fig. 1d). Importantly, $TSG101^+$ exosomes from plasma of NDs also carried LAAs and $DPP4^+$, although the levels of these proteins were lower than those in AML exosomes (Fig. 1d).

DPP4 is present on exosomes and is enzymatically active

Flow cytometry of exosomes isolated from the Kasumi cell line, AML patients' plasma, and ND's plasma, and captured on magnetic beads for antigen detection confirmed the presence of DPP4 on the surface of exosomes (Fig. 1e). Quantitatively, AML exosomes carried significantly higher levels of DDP4 than ND's exosomes. DPP4 carried on exosomes was evaluated for enzymatic activity as described in Methods. The data in Fig. 2a show that exosomes isolated from AML patients' plasma or leukemia cell lines had significantly higher levels of DPP4 activity than those measured in the exosomes of NDs. When Diprotin A, a DPP4 inhibitor, was added to exosomes, it significantly ($p < 0.01$–0.05) reduced enzymatic DPP4 activity (Fig. 2b).

Effects of leukemia exosomes on the colony formation of normal HPC

Multi-potential (CFU-GEMM) and granulocyte macrophage (CFU-GM) colony forming cell assays were performed with human cord blood in the presence of cytokines and exosomes isolated from the plasma of AML patients, ND's plasma, and supernatants of leukemia cell lines. AML plasma-derived exosomes significantly ($p < 0.01$–0.05) suppressed the colony formation of normal HPC (Fig. 3a). AML-derived exosomes showed significantly higher suppression of colony formation than the exosomes of NDs. The suppression levels of CFU-GEMM and CFU-GM formation by AML-derived exosomes were approximately the same. The data in Fig. 3b indicate that this suppression was exosome dose-dependent.

Diprotin A reverses effects of leukemia exosomes on colony formation

Since enzymatically active DPP4 was present on the surface of AML-derived exosomes and Diprotin A was shown to inhibit its activity, we next investigated whether inhibition of DPP4 activity reduced the exosome-mediated suppression of HPC differentiation. As illustrated in Fig. 3c, Diprotin A mitigated the suppressive effects of exosomes on colony formation ($p < 0.01$–0.05). The inhibitory effects of Diprotin on the CFU-GEMM and CFU-GM were comparable.

Exosomes from CR patients' plasma suppresses HPC differentiation

Having demonstrated that exosomes from the plasma of untreated AML patients at diagnosis suppressed colony formation beyond that of ND's exosomes, we next examined plasma-derived exosomes of AML patients who had achieved complete remission (CR) or incomplete remission (CRi, defined as <100,000/μL platelets or <1000/μL neutrophils measured in the peripheral blood) [29] using colony inhibition assays.

We expected to observe differences in the levels of HSC suppression by exosomes from plasma of CR *versus* CRi patients. Exosomes were isolated from paired samples of plasma obtained at diagnosis and at the time of CR ($n = 5$) or CRi ($n = 5$) following induction chemotherapy. Exosomes from CRi patients' plasma suppressed in vitro colony formation as much as did plasma exosomes obtained at AML diagnosis. Thus, in paired assays and after adjusting for exosome protein levels used for the assays, suppression of colony formation mediated by CRi exosomes remained as high as that mediated by plasma exosomes obtained at AML diagnosis (Fig. 4). In contrast, levels of suppression mediated by exosomes of AML patients who achieved CR were significantly decreased compared to those observed at AML diagnosis (Fig. 4). Importantly, DPP4 activity in CRi patients' exosomes remained at levels that were similar to DDP4 levels in the exosomes obtained at AML diagnosis ($p < 0.1$). In contrast, levels of DPP4 activity in exosomes of patients in CR were significantly lower ($p < 0.04$) compared to DPP4 activity in exosomes at AML diagnosis.

Discussion

AML is one of the most common hematologic malignancies, and its mortality and morbidity are largely due to frequently occurring cytopenias. The associated cytopenias have been attributed to the secretion of negative regulatory cytokines by leukemic cells in the BM, the replacement of normal HSC by leukemic cells, and remodeling of the BM niche to favor leukemic cell survival or growth over normal hematopoietic cells [2]. In the current study, we implicate AML blast-derived $DPP4^+$ exosomes in the suppression of normal hematopoiesis that could contribute to cytopenias in AML.

Emerging studies of exosomes in hematologic malignancies are uncovering novel, previously unsuspected, aspects of AML pathogenesis. Collectively, these studies unveil exosome-mediated mechanisms that operate in leukemia and might be involved in the suppression of normal hematopoiesis. The suppression of HPC functions is known to be partially mediated through stromal reprogramming of niche-retention factors and also as a consequence of AML exosome-directed miRNA delivery to HPC [13]. In the current study, we demonstrated that exosomes isolated from the plasma of AML patients carry abundant enzymatically active DPP4 and suppress normal human HPC differentiation in vitro. The specificity of this suppression is documented by the fact that pharmacological DPP4 inhibition reversed the exosome-mediated suppression of colony formation. Furthermore, we have shown that the plasma of AML patients who achieved morphologic CR after chemotherapy but had incomplete cell count recovery contained high plasma levels of exosomes that were myelosuppressive in vitro.

Treatment of patients with AML aims at achieving CR and at restoration of normal BM functions.

However, some AML patients who achieve CR following induction chemotherapy have incomplete count recovery (CRi, <100,000/μL platelets or <1000/μL neutrophils in the peripheral blood) [29]. Patients with CR fare better than those with CRi and have better relapse-free survival and overall survival [30].

Factors associated with impaired hematopoietic recovery in AML following therapy are largely unknown. Recent data in pediatric populations suggest that lower telomere content at the end of AML induction predicts delays in the recovery of absolute neutrophil counts during later chemotherapy courses, suggesting that telomere content reflects the stress of serial courses of intensive chemotherapy on the stem cell pool [31]. The contribution of other recognized stressors, such as infection or the production of pro-inflammatory cytokines, may also contribute to differences in the reconstitution time amongst individuals with AML.

We previously reported that DPP4-truncated GM-CSF and IL-3 had diminished colony-stimulating activity and intracellular signaling compared to their full-length forms, and that inhibition of DPP4 enhanced the activity of GM-CSF and IL-3 [26]. Blocking DPP4 in AML exosomes partially or completely mitigated their suppressive effects on colony formation in vitro. It is possible that reversing the myelosuppressive effects of $DPP4^+$ exosomes may enhance the count recovery in CRi patients. Such an approach in AML patients is clinically feasible, as systemic use of an oral DPP4 inhibitor, sitagliptin, enhanced neutrophil engraftment of single-unit umbilical cord transplantation in patients with high risk hematological malignancies [32, 33].

Our study represents the first analysis of DPP4-carrying exosomes isolated from the plasma of patients with AML at various time points in the course of therapy. The elevated levels of these exosomes in AML plasma and of exosome-associated DPP4 activity are related to exosome-mediated suppression of normal HPC colony formation and, by extension, of cell count recovery.

Interestingly, in this study plasma exosome levels remained significantly elevated in patients achieving morphological remission, and plasma exosomes in CRi patients mediated significantly elevated myelosuppression. Whether these myelosuppressive exosomes in the plasma of CR or CRi patients are derived from reprogrammed normal cells or from minimal residual leukemia blasts remains unknown.

In conclusion, our report that exosomes in the plasma from AML patients inhibit normal human hematopoiesis in vitro, implicates exosome-associated DPP4 in this suppression mechanism. The data suggest that, in the future, reversing the negative effects of exosomes and thus improving platelet and neutrophil counts, might emerge as a new therapeutic approach to AML. However, such a clinical course would need to be cautiously considered due to the various regulatory proteins that contain DPP4 truncation sites and might exert different inducing, enhancing, or suppressive effects on hematopoiesis [24–26, 34].

Funding

This work was supported by the U.S National Cancer Institute grant R21 CA205644 to TLW and MB and by NIH R35 HL139599 to HB.

References

1. Short NJ, Rytting ME, Cortes JE. Acute myeloid leukaemia. Lancet. 2018;392:593–606. [PubMed: 30078459]
2. Cheng H, Cheng T. 'Waterloo': when normal blood cells meet leukemia. Curr Opin Hematol. 2016;23:304–10. [PubMed: 27115340]
3. Miraki-Moud F, Anjos-Afonso F, Hodby KA, Griessinger E, Rosignoli G, Lillington D, et al. Acute myeloid leukemia does not deplete normal hematopoietic stem cells but induces cytopenias by impeding their differentiation. Proc Natl Acad Sci USA. 2013; 110:13576–81. [PubMed: 23901108]
4. Cheng H, Hao S, Liu Y, Pang Y, Ma S, Dong F, et al. Leukemic marrow infiltration reveals a novel role for Egr3 as a potent inhibitor of normal hematopoietic stem cell proliferation. Blood. 2015;126:1302–13. [PubMed: 26186938]
5. Szczepanski MJ, Szajnik M, Welsh A, Whiteside TL, Boyiadzis M. Blast-derived microvesicles in sera from patients with acute myeloid leukemia suppress natural killer cell function via membrane-associated transforming growth factor-beta1. Haema-tologica. 2011;96:1302–9.
6. Hong CS, Muller L, Whiteside TL, Boyiadzis M. Plasma exosomes as markers of therapeutic response in patients with acute myeloid leukemia. Front Immunol. 2014;5:160. [PubMed: 24782865]
7. Boyiadzis M, Whiteside TL. The emerging roles of tumor-derived exosomes in hematological malignancies. Leukemia. 2017;31: 1259–68. [PubMed: 28321122]
8. Shah R, Patel T, Freedman JE. Circulating extracellular vesicles in human disease. N. Engl J Med 2018;379:958–66. [PubMed: 30184457]
9. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014;29:116–25. [PubMed: 24959705]
10. Dreyer F, Baur A. Biogenesis and functions of exosomes and extracellular vesicles. Methods Mol Biol. 2016;1448:201–16. [PubMed: 27317183]
11. Kalluri R The biology and function of exosomes in cancer. J Clin Investig. 2016;126:1208–15. [PubMed: 27035812]
12. Hong CS, Sharma P, Yerneni SS, Simms P, Jackson EK, Whiteside TL, et al. Circulating exosomes carrying an immunosuppressive cargo interfere with cellular immunotherapy in acute myeloid leukemia. Sci Rep. 2017;7:14684. [PubMed: 29089618]
13. Boyiadzis M, Whiteside TL. Exosomes in acute myeloid leukemia inhibit hematopoiesis. Curr Opin Hematol. 2018;25:279–84. [PubMed: 29846239]
14. Hornick NI, Doron B, Abdelhamed S, Huan J, Harrington CA, Shen R, et al. AML suppresses hematopoiesis by releasing exosomes that contain microRNAs targeting c-MYB. Sci Signal. 2016;9:ra88. [PubMed: 27601730]
15. Huan J, Hornick NI, Shurtleff MJ, Skinner AM, Goloviznina NA, Roberts CT Jr., et al. RNA trafficking by acute myelogenous leukemia exosomes. Cancer Res. 2013;73:918–29. [PubMed: 23149911]
16. Kumar B, Garcia M, Weng L, Jung X, Murakami JL, Hu X, et al. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. Leukemia. 2018;32:575–87. [PubMed: 28816238]
17. Sato A, Ogita H. Pathophysiological implications of dipeptidyl peptidases. Curr Protein Pept Sci. 2017;18:843–9. [PubMed: 28356045]
18. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocr Rev. 2014;35:992–1019. [PubMed: 25216328]
19. Klemann C, Wagner L, Stephan M, von Horsten S. Cut to the chase: a review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. Clin Exp Immunol. 2016;185: 1–21. [PubMed: 26919392]

20. Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. Pharmacol Rev. 2008;60:470–512. [PubMed: 19074620]
21. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol. 2019;10:80.
22. Gallwitz B Clinical use of DPP-4 inhibitors. Front Endocrinol. 2019;10:389.
23. Beckenkamp A, Davies S, Willig JB, Buffon A. DPPIV/CD26: a tumor suppressor or a marker of malignancy? Tumour Biol. 2016;37:7059–73. [PubMed: 26943912]
24. Broxmeyer HE, Hoggatt J, O'Leary HA, Mantel C, Chitteti BR, Cooper S, et al. Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis. Nat Med. 2012;18:1786–96. [PubMed: 23160239]
25. Ou X, O'Leary HA, Broxmeyer HE. Implications of DPP4 modification of proteins that regulate stem/progenitor and more mature cell types. Blood. 2013;122:161–9. [PubMed: 23637126]
26. O'Leary HA, Capitano M, Cooper S, Mantel C, Boswell HS, Kapur R, et al. DPP4 truncated GM-CSF and IL-3 manifest distinct receptor-binding and regulatory functions compared with their full-length forms. Leukemia. 2017;31:2468–78. [PubMed: 28344320]
27. Hong CS, Funk S, Muller L, Boyiadzis M, Whiteside TL. Isolation of biologically active and morphologically intact exosomes from plasma of patients with cancer. J Extracell vesicles. 2016;5:29289. [PubMed: 27018366]
28. Theodoraki MN, Hong CS, Donnenberg VS, Donnenberg AD, Whiteside TL. Evaluation of exosome proteins by on-bead flow cytometry. Cytometry A. 2020;24193.
29. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21:4642–9. [PubMed: 14673054]
30. Walter RB, Kantarjian HM, Huang X, Pierce SA, Sun Z, Gun-dacker HM, et al. Effect of complete remission and responses less than complete remission on survival in acute myeloid leukemia: a combined Eastern Cooperative Oncology Group, Southwest Oncology Group, and M. D. Anderson Cancer Center Study. J Clin Oncol. 2010;28:1766–71. [PubMed: 20159819]
31. Gerbing RB, Alonzo TA, Sung L, Gamis AS, Meshinchi S, Plon SE, et al. Shorter remission telomere length predicts delayed neutrophil recovery after acute myeloid leukemia therapy: a report from the children's oncology group. J Clin Oncol. 2016;34: 3766–72. [PubMed: 27354474]
32. Farag SS, Nelson R, Cairo MS, O'Leary HA, Zhang S, Huntley C, et al. High-dose sitagliptin for systemic inhibition of dipeptidylpeptidase-4 to enhance engraftment of single cord umbilical cord blood transplantation. Oncotarget. 2017;8: 110350–7. [PubMed: 29299152]
33. Farag SS, Srivastava S, Messina-Graham S, Schwartz J, Robert-son MJ, Abonour R, et al. In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. Stem Cells Dev. 2013;22:1007–15. [PubMed: 23270493]
34. Broxmeyer HE, Capitano M, Campbell TB, Hangoc G, Cooper S. Modulation of hematopoietic chemokine effects in vitro and in vivo by DPP-4/CD26. Stem Cells Dev. 2016;25:575–85. [PubMed: 26943017]

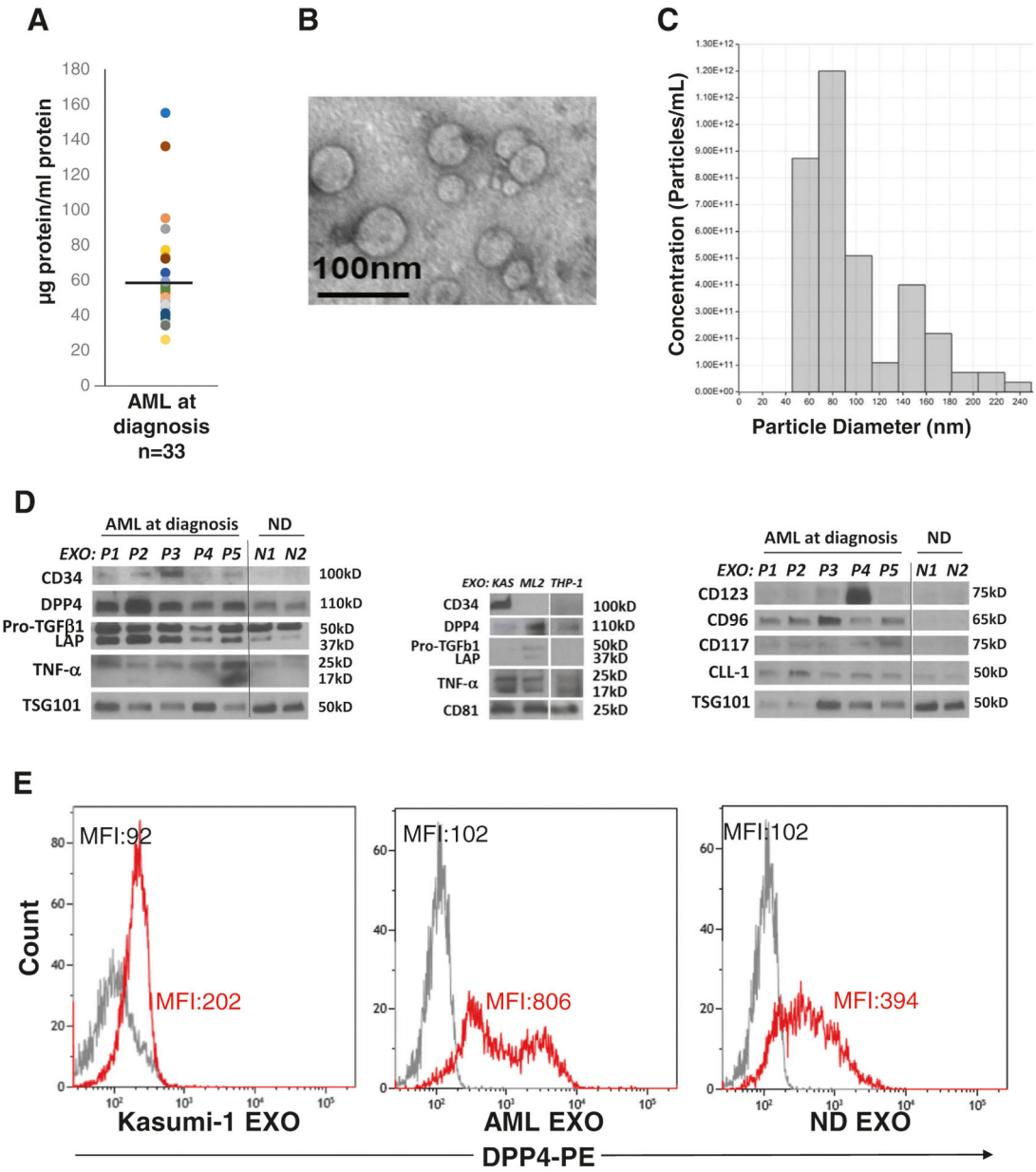

Fig. 1. Characteristics and molecular profile of isolated exosomes.

a Mean protein levels in exosomes isolated from the plasma of AML patients at diagnosis (n = 33) prior to any therapy. **b** Transmission electron microscopy of the isolated exosomes. **c** Size and concentration of the exosomes as determined by tunable resistive sensing using q-Nano. **d** Molecular profiles of AML exosomes isolated by mini-SEC from the pre-cleared plasma of AML patients or the supernatants of leukemia cell lines. **e** To investigate whether DPP4 is present on the surface of exosomes on-bead flow cytometry was performed demonstrating the presence of DPP4 on the surface of exosomes isolated from Kasumi-1 cell line supernatant, plasma of an AML patient, and plasma of a ND. MFI: mean fluorescence intensity, gray: isotype control, red: anti-CD26/DPP4. The flow cytometry experiments were repeated with three different exosomes obtained from patients, NDs or Kasumi-1 cultures.

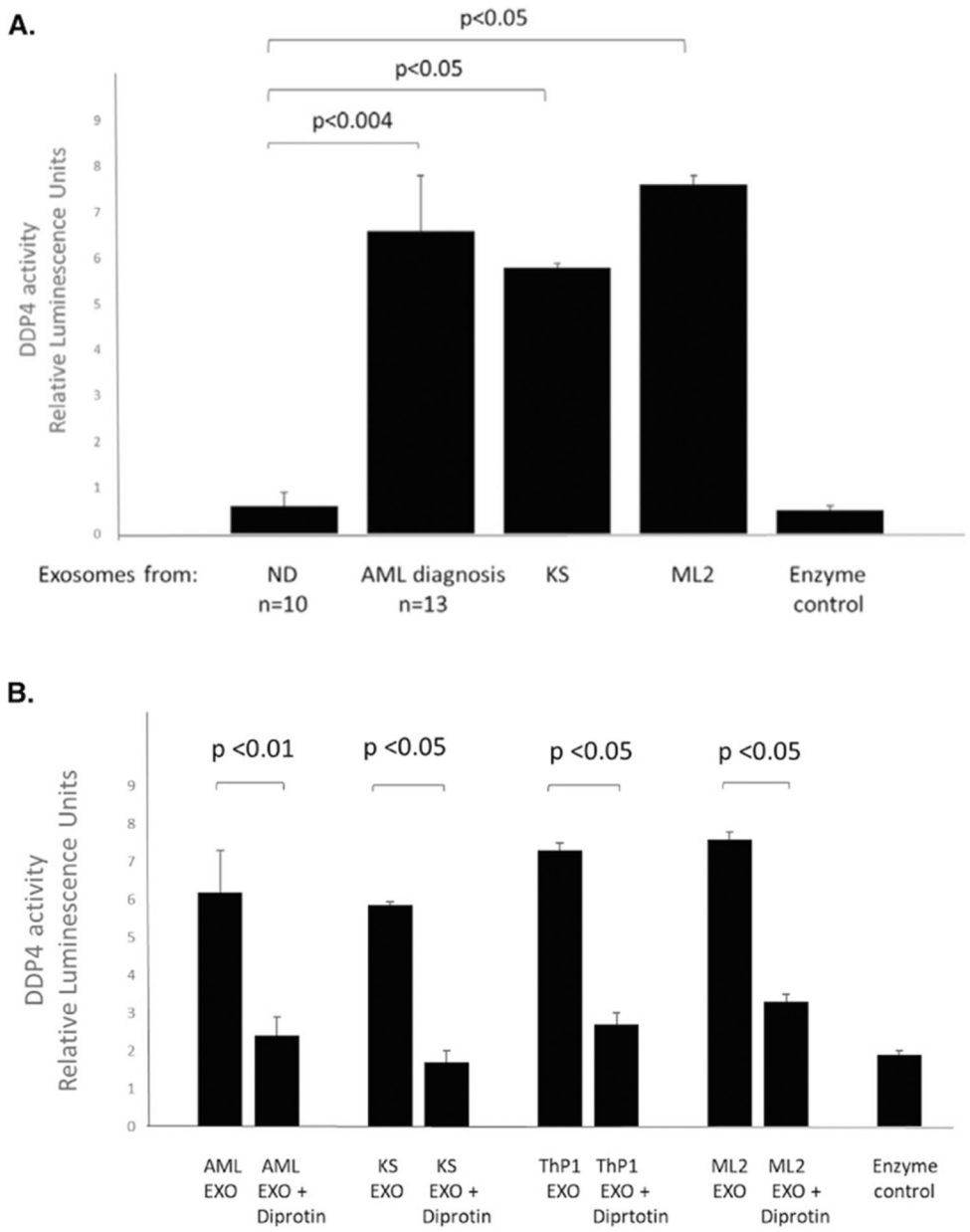

Fig. 2. DPP4 in leukemia exosomes is enzymatically active.

a Exosomes (10 μg protein) isolated from the plasma of AML patients at diagnosis (n = 13), supernatants of leukemia cell lines (Kasumi-1, ML2), and from plasma of NDs (n = 10) were plated and combined with the DPP4 substrate. Exosomes isolated from AML patients and leukemia cell lines exhibited a markedly higher DPP4 activity than that seen in ND's exosomes. **b** Diprotin A, a DPP4 inhibitor, was added prior to the assay to show suppression of DPP4 activity in exosomes. Exosomes were isolated from plasma of five AML patients and from supernatants of leukemia cell lines. The same exosome concentration was used for the performed experiments, as described in Materials and Methods.

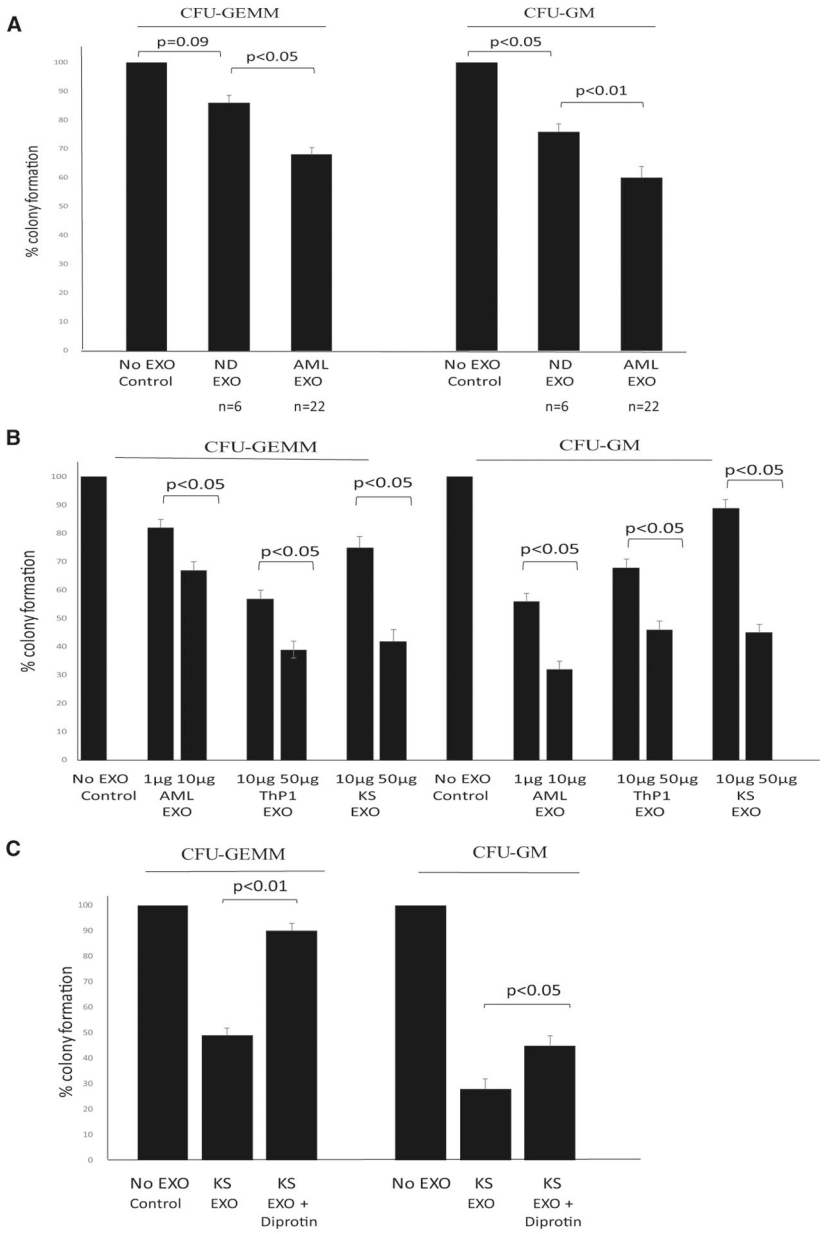

Fig. 3. Effects of leukemia exosomes on the proliferation of normal hematopoietic progenitor cells.

a Leukemia exosomes block colony formation. Exosomes (10 μg protein) isolated from the plasma of AML patients at diagnosis ($n = 22$) and normal donors ($n = 6$) were suspended in PBS and plated in 35 mm cultures dishes. Cells were plated in triplicate. The mean control colony numbers for CFU-GEMM and CFU-GM were 54 and 53, respectively. **b** Inhibition of colony formation by leukemia exosomes was dose dependent. Cells were plated in triplicates. The mean control colony numbers for CFU-GEMM and CFU-GM were 81 and 68, respectively. **c** Diprotin A reversed the inhibitory effects of exosomes on colony formation. For reversal of inhibition experiments, 10 μg exosomes/well were combined with 0.2 mmol Diprotin A for 4 h prior to plating. The inhibitory effects of Diprotin A were comparable for CFU-GEMM and CFU-GM colony formation.

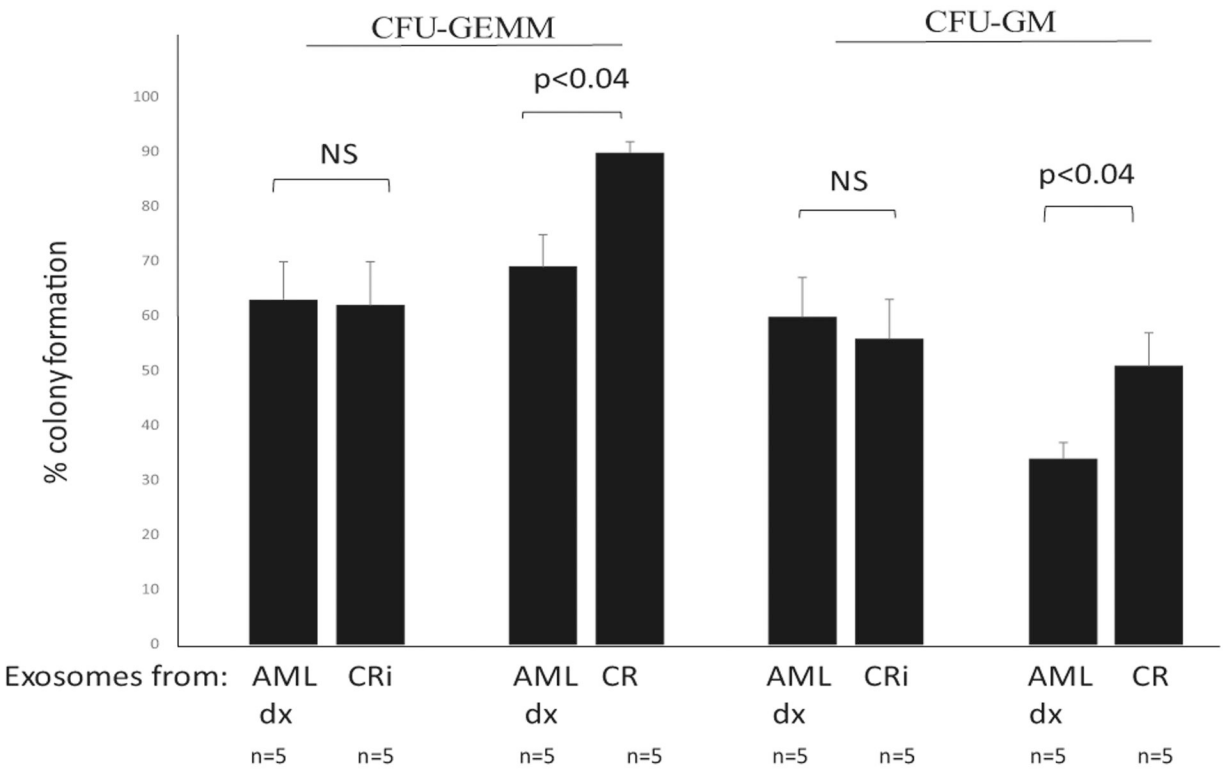

Fig. 4. Effects of leukemia exosomes from patients in complete remission.

Exosomes from AML patients who achieved CRi or CR had different effects on colony formation. Paired samples were collected at AML diagnosis and at CR ($n = 5$) and CRi ($n = 5$). Exosomes from CRi patients suppressed in vitro colony formation whereas the degree of suppression by the exosomes of AML patients who achieved CR decreased compared to that at AML diagnosis. NS not statistically significant.