Title: DEK, a Nuclear Protein, is Chemotactic for Hematopoietic Stem/Progenitor Cells Acting Through CXCR2 and Gαi Signaling

Authors: Maegan L. Capitano[*], Yasser Sammour[*], James Ropa[*], Maureen Legendre[†], Nirit Mor-Vaknin[†], and David M. Markovitz[†]
Authors Affiliations: [*]Indiana University School of Medicine, Department of Microbiology and Immunology, Indianapolis, Indiana, USA; [†]Department of Internal Medicine, Division of Infectious Diseases, University of Michigan, Ann Arbor, Michigan, USA.
Correspondence To: Maegan L. Capitano, Ph.D.
Department of Microbiology and Immunology
Indiana University School of Medicine
950 West Walnut Street, R2-302
Indianapolis, IN 46202
Phone: (317) 274-7555
E-mail: mlcapit@iupui.edu
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Summary Sentence: The nuclear protein DEK acts as a chemotactic factor for hematopoietic stem and progenitor cells in a CXCR2- and Gαi protein-coupled signaling-dependent manner.
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Abbreviations:
HSC: hematopoietic stem cell

HPC: hematopoietic progenitor cell

Lin$^-$: lineage negative

BM: bone marrow

LSK cells: Lin$^-$ Sca-1$^+$ c-Kit$^+$ cells

ELR motif: Glu-Leu-Arg motif

CK2: casein kinase 2

C/EBPα: CCAAT enhancer binding protein α

EKLF: erythroid Kruppel-like factor

IL-8: interleukin-8

MIP2: macrophage inflammatory protein 2

AKT: protein kinase B

MAPK: mitogen-activated protein kinase

SDF1α: stromal cell-derived factor 1α

IUSM: Indiana University School of Medicine

rm: recombinant mouse

rh: recombinant human

TPO: thrombopoietin

Flt3L: Flt3 ligand

PT: Pertussis toxin

LT: long-term

ST: short-term

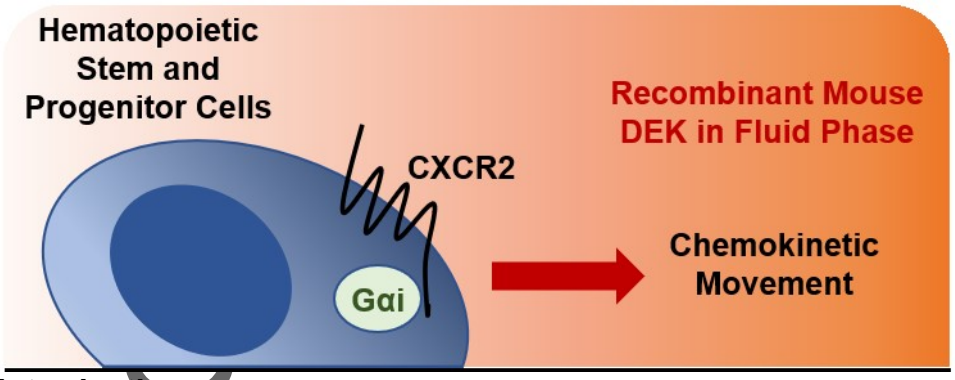
MPP: multipotent progenitor cells

KC: keratinocyte-derived chemokine

Abstract

Few cytokines/growth modulating proteins are known to be chemoattractants for hematopoietic stem (HSC) and progenitor cells (HPC); stromal cell-derived factor 1α (SDF1α/CXCL12) being the most potent known such protein. DEK, a nuclear DNA-binding chromatin protein with hematopoietic cytokine-like activity, is a chemotactic factor attracting mature immune cells. Transwell migration assays were performed to test whether DEK serves as a chemotactic agent for HSC/HPC. DEK induced dose- and time-dependent directed migration of lineage negative (Lin^-) $Sca\text{-}1^+$ $c\text{-}Kit^+$ (LSK) bone marrow (BM) cells, HSCs and HPCs. Checkerboard assays demonstrated that DEK's activity was chemotactic (directed), not chemokinetic (random migration), in nature. DEK and SDF1α compete for HSC/HPC chemotaxis. Blocking CXCR2 with neutralizing antibodies or inhibiting Gαi protein signaling with Pertussis toxin pretreatment inhibited migration of LSK cells towards DEK. Thus, DEK is a novel and rare chemotactic agent for HSC/HPC acting in a direct or indirect CXCR2 and Gαi protein-coupled signaling-dependent manner.

Graphical Abstract: The nuclear protein DEK acts as a chemotactic factor for hematopoietic stem and progenitor cells in a CXCR2- and GÎ±i proteincoupled signaling-dependent manner.



Introduction

Nuclear protein DEK, a non-histone chromosomal factor, is vital for global heterochromatin integrity, transcription, DNA repair and gene regulation[1-3], and post-translational modifications of DEK greatly influence its function.[4-7] Disassociation of phosphorylated DEK from chromatin, and thus the nucleus, allows its secretion by hematopoietic cells in free-form or in exosomes by IL-8-stimulated monocyte-derived macrophages in a CK2-dependent and Golgi-apparatus-independent manner.[8,9] Poly-ADP-ribosylation of DEK allows for its passive secretion by T cells undergoing apoptosis.[7] Secreted DEK is associated with autoimmune diseases such as juvenile idiopathic arthritis, sarcoidosis, and systemic lupus erythematosus.[2,9,10] In autoimmune arthritis, auto-antibodies against DEK and DEK itself are detected in synovial fluid of arthritic joints and are required for maximum inflammatory cell recruitment into joint tissue.[9,11,12] Secreted DEK, in free-form, is a chemoattractant for neutrophils, $CD8^+$ T lymphocytes, and natural killer cells.[9,11]

Both endogenous DEK and extracellular, recombinant DEK regulate hematopoiesis.[2,9,13-16] Endogenous, nuclear DEK is required for the optimal function of the CCAAT enhancer binding protein (C/EBP)α, a transcription factor that coordinates proliferation arrest and myeloid progenitor cell differentiation into mature myeloid cells.[17] DEK also interacts with upstream enhancer elements of the erythroid

Kruppel-like factor (EKLF) promotor, increasing expression of EKLF, a zinc finger transcription factor that plays a role in the global regulation of erythroid gene expression.[18-22] Thus, endogenous DEK plays a role by maintaining HSC function and regulating myelopoiesis. Moreover, extracellular, recombinant DEK regulates hematopoiesis, enhances *ex vivo* expansion of functional mouse and human HSC, and increases HSC numbers with subsequent decreases in HPC numbers and cycling.[16]

Very few proteins, SDF1α/CXCL12 being the most potent, have chemotactic activity for HSC/HPC.[23,24] We have shown that extracellular DEK suppresses HPC proliferation through a CXCR2-dependent mechanism similarly to the chemokines IL-8 (only expressed in humans) and MIP2.[9,16,25-27] DEK activates, either directly or indirectly, a CXCR2 signaling cascade in HSC and HPC involving Gαi, ERK, protein kinase B (AKT), and p38 mitogen-activated protein kinase (MAPK).[16] Since DEK is chemotactic for multiple mature hematopoietic cells and regulates hematopoiesis in a CXCR2-dependent manner[9,16], we hypothesized that DEK could act as a chemotactic protein for HSC and HPC. We now show that extracellular DEK acts as a rare chemotactic agent for HSC/HPC in a CXCR2- and Gαi-dependent manner and competes with SDF1α/CXCL12 in mediating HSC/HPC migration.

Materials and Methods

Mice. C57BL/6J mice (6-10 weeks old) obtained from an on-site breeding core facility at Indiana University School of Medicine (IUSM) were maintained under temperature- and light-controlled conditions (21–24°C, 12-hour light/12-hour dark cycle) and were group-housed according to age and sex, fed *ad libitum*, and matched by age and sex for all experiments. All animal experiments followed protocols approved by the Institutional Animal Care and Use Committee of IUSM.

RNA-seq analysis. Raw RNA-sequencing reads have previously been deposited in the Gene Expression Omnibus (GEO) under accession GSE126875. Reads were aligned and assigned to the mouse genome (mm10) using STAR and HTSeq as previously described.[16] Gene counts were normalized by library size and differential expression analysis was performed comparing mouse Lin^- BM treated with recombinant mouse (rm)DEK compared to vehicle treated cells using DESeq2 R package with the design ~mouse+treatment. Fast gene set enrichment (FGSEA) was performed using fgsea R package to compare ranked gene list using the test statistic from DESeq2 to rank gene expression differences to *a priori* defined gene sets from the MSigDB. Fgsea was performed with the following parameters: pathways = c(Mm.C2,Mm.C5,Mm.C6); nperm=1000; minSize=25; maxSize=500. For examining *CXCR2* expression levels in mice and humans, microarray normalized log2 expression value data was downloaded from the BloodSpot (https://servers.binf.ku.dk/bloodspot/) database and plotted in GraphPad Prism. Normalization for these data has been previously described.[28]

BM Lin^- and $Ly6G^+$ cell chemotaxis. RmDEK was purified from insect cells as described previously.[5,14,16] Recombinant DEK was dialyzed prior to its use. BM Lin^- and $Ly6G^+$ cells were prepared as described in Supplemental Materials and Methods. Costar 24-well transwell plates with 6.5 mm diameter inserts with 5.0 μm pores (Corning Inc, Corning, NY, USA) were used for chemotaxis assays. 650 μL pre-warmed serum free IMDM medium containing recombinant human (rh)SDF1α (R&D Systems; catalog #350-NS), rmDEK, rhIL-8/CXCL8 (R&D Systems, catalog 208-IL) or rmMIP2/CXCL2 (R&D Systems, catalog #452-M2) at the indicated

concentrations were added to the lower and/or upper chamber as indicated. Media alone served as a negative control. Mouse BM Lin^- or $Ly6G^+$ cells (1×10^5 cells/100 µL) were resuspended in IMDM with 0.5% bovine serum albumin (BSA; Sigma-Aldrich, Miamisburg, OH, USA). Cell suspensions (100 µL) were placed in the upper chamber of the transwell plate. Transwell plates were placed in a 37 °C incubator with 5% CO_2 and 95% humidity for 4 hours or indicated time points. Percent migration was determined by flow cytometry as described in Supplemental Materials and Methods. To examine the importance of Gαi protein–coupled receptor signaling in DEK-mediated chemotaxis of BM Lin^- and $Ly6G^+$ cells, we incubated Lin^- and $Ly6G^+$ cells from C57BL/6 mice with 1000 ng/mL Pertussis toxin (PT; Sigma-Aldrich; catalog P7208) for 4 hours at 37°C immediately prior to the chemotaxis assay. To block CXCR2 and CXCR4 on the cell surface, BM Lin^- and $Ly6G^+$ cells were incubated with 2.5 µg/10^6 cells of anti-mouse CXCR2 purified rat monoclonal IgG2A antibody (R&D Systems, clone 242216), anti-mouse CXCR4 purified rat monoclonal IgG2B antibody (R&D Systems, clone 247506), or isotype rat IgG control (azide-free; R&D Systems, catalog 6-001-F) for 30 minutes at room temperature prior to use and cells washed.

Statistics. Results are expressed as mean values ± standard deviation. Two-tailed Student's t test was used where indicated. One way ANOVA with post-hoc Tukey's multiple-comparisons test was used when comparing 3 or more groups. Statistical analysis was performed using Microsoft Excel and GraphPad Prism 5.0. $P < 0.05$ was considered statistically significant.

Results/Discussion

HSC/HPC-enriched LSK cells migrate towards rmDEK in a time- and dose-dependent manner. To examine novel functions of rmDEK in HSC and HPC regulation, we re-examined RNA-seq data from our previous study demonstrating that DEK regulates hematopoiesis.[16] A deeper look at this RNA-seq data of pooled mouse BM HSCs and HPCs treated with rmDEK overnight compared to vehicle control treated cells revealed that gene programs associated with chemotaxis are significantly upregulated upon treatment with rmDEK (Figure 1A). This includes genes associated broadly with cell chemotaxis, genes associated specifically with leukocyte migration, and genes that positively regulate cell-cell adhesion. It is well known that DEK is secreted by macrophages and acts as a proinflammatory molecule serving as a chemotactic factor attracting neutrophils, $CD8^+$ T lymphocytes and natural killer cells.[7,9,12] Very few cytokines/chemokines chemotax HSCs/HPCs, SDF1α/CXCL12 being the most potent of such proteins.[23,24] To test if extracellular DEK can chemotax HSCs/HPCs, transwell migration assays were performed utilizing Lin^- BM cells. LSK cells (enriched in HSCs/HPCs) within the Lin^- BM cell population migrated towards 100 ng/mL rmDEK with maximum percent migration reached by 4 hours in culture (Figure S1A). LSK cells migrated towards rmDEK in a dose-dependent manner, with maximum percent migration (~20%) occurring at equal to or greater than 50 ng/mL rmDEK following both 4 (Figure S1B) and 8 hour (Figure S1C) incubation.

rmDEK induces chemotactic, not chemokinetic, movement of LSK cells. Not all factors that influence cellular migration do so in a directional, non-random, way (e.g., stem cell factor is chemokinetic for HPC).[24] To determine if DEK mediates

chemotactic (directional migration towards a chemoattractant gradient) or chemokinetic (random migration) movement of LSK cells, we performed a checkerboard analysis of LSK cell migration (Fig. S2A&B; Tables 1 and 2). Checkerboard analysis was performed by placing 0-100 ng/mL of rmDEK in the bottom well and 0-100 ng/mL of rmDEK in the insert (top well) of the transwell assay (Fig. S2A) so that there are wells with different concentrations of rmDEK on the top and bottom compartments (e.g., 0 ng/mL on top:50 ng/mL on bottom, 50 ng/mL on top:50 ng/mL on bottom, etc.). If cells still migrate to the bottom well when there is rmDEK in the top well then the movement is considered random, thus rmDEK most likely would be producing chemokinetic movement. When Lin^- cells were placed into media alone in the top well, LSK cells migrated towards the bottom chambers that contained 50 and 100 ng/mL rmDEK (Table 1). However, when rmDEK was added to the top well, LSK cell migration was significantly inhibited suggesting that rmDEK mediates chemotactic, not chemokinetic movement, of LSK cells. To confirm that our checkerboard assays were accurate, we repeated this procedure utilizing rhSDF1α as a positive control and confirmed that LSK cell migration towards SDF1α is chemotactic (Fig. S2B and Table 2).

rmDEK induces migration of LT-HSC, ST-HSC, and MPP populations. To determine for which HSC and HPC populations rmDEK acts as a chemoattractant, we first examined LSK and LK (a myeloid progenitor-enriched) cell migration towards rmDEK and rhSDF1α, the latter as a positive control. LSK and LK cell populations migrated towards rhSDF1α and rmDEK (Figure 1B&C). Within the LSK population are the long-term (LT)-HSC, short-term (ST)-HSC and multipotent progenitor (MPP) cell

populations. LT-HSC, ST-HSC, and MPP all migrated towards rmDEK and rhSDF1α (Fig. 1D).

DEK is a more potent chemoattractant for LSK cells than is SDF1α. Since both rmDEK and rhSDF1α induce LSK migration in transwell migration assays, we examined whether one or the other is a more potent chemoattractant when in competition with each other. Checkboard assays were performed where different concentrations of rmDEK were used in the top well and different concentrations of rhSDF1α were used on the bottom (Fig. S2C and Table 3) or vice versa (Fig. S2D and Table 4). Since rhSDF1α is an 8 kDa protein and rmDEK is a 43-50 kDa protein, we performed these checkerboard assays using molarity. The addition of 2.5 nM rmDEK to the top insert/well resulted in significant inhibition of LSK cell migration towards 10 nM SDF1α (Fig. S2C and Table 3). However, it took 10nM SDF1α to significantly inhibit LSK cell migration towards 10nM rmDEK (Fig. S2D and Table 4) suggesting DEK is a more potent chemoattractant for LSK cells.

LSK cell migration towards rmDEK is CXCR2- and Gαi protein-coupled signaling-dependent. Because DEK requires the chemokine receptor CXCR2 to regulate hematopoiesis[9,16], we hypothesized that DEK may manifest its chemotactic actions through a CXCR2-dependent mechanism. First, CXCR2 expression was examined in various subpopulations of human and mouse hematopoietic cells utilizing the publicly available microarray data compiled by BloodSpot database.[28-36] These analyses revealed that while there is generally more CXCR2 RNA expressed in mature myeloid cells and HPC populations, CXCR2 is also expressed at detectable levels in human HSC and mouse LT-HSC and ST-HSC (Fig. S3). Next,

we performed migration assays where LSK cells were pretreated with neutralizing monoclonal antibody for CXCR2 immediately prior to being placed in the upper chamber of a transwell chemotaxis assay utilizing 100 ng/mL of rmDEK in the bottom chamber. $Ly6G^+$ cells (neutrophils) were utilized as a positive control as they migrate towards SDF1α via CXCR4, MIP2 via CXCR2, IL-8 via CXCR1/CXCR2 and DEK.[9,37-40] Neutralizing anti-CXCR2 antibodies inhibited migration of both LSK and $Ly6G^+$ cells toward rmDEK; however, if LSK cells were pretreated with an isotype control or a neutralizing antibody towards CXCR4, migration towards DEK was not blocked (Fig. 2A). To confirm that the neutralizing CXCR2 antibody did not inhibit migration in a non-specific manner, transwell assays were performed examining LSK cell migration towards rhSDF1α, rhIL-8, and rmMIP2. LSK cells were still able to migrate towards rhSDF1α except when CXCR4 was neutralized. As previously reported, no migration of LSK cells was observed when IL-8 or MIP2 was utilized.[41,42] When $Ly6G^+$ neutrophils were used, CXCR2 neutralizing antibodies blocked migration of the $Ly6G^+$ neutrophils towards rmDEK, rhIL-8 and rmMIP2 (Fig. 2B). Neutralizing CXCR4 only blocked $Ly6G^+$ neutrophil migration towards rhSDF1α. Chemokine receptors couple to G proteins for signal transduction and this interaction can be blocked using Pertussis toxin (PT), which prevents Gαi proteins from interacting with G protein-coupled receptors and thus interfering with receptor signaling.[43] Pretreatment of LSK cells with PT significantly inhibited migration of LSK cells towards rmDEK and rhSDF1α (Fig. 2C). Pretreatment of $Ly6G^+$ neutrophils with PT resulted in significant reduction in migration towards rhSDF1α, rmDEK, rhIL-8 and rmMIP2 (Fig. 2D). These data taken together demonstrate that LSK cell-directed migration toward rmDEK is CXCR2- and G protein-coupled signaling dependent.

We now demonstrate that extracellular DEK induces LSK cell (e.g., LT-HSC, ST-HSC, and MPP) migration in a dose- and time-dependent, chemotactic manner. It is striking that this nuclear protein, when extracellular, can have such profound effects not only in regulating hematopoiesis[14-16], but by acting as a chemotactic agent for HSC/HPC as well. Like other chemokines (e.g., IL-8 and MIP2), DEK suppresses functional HPC numbers in a CXCR2-dependent manner.[16,44-47] DEK-mediated enhancement in HSC numbers *in vivo* and *ex vivo* is also dependent on CXCR2.[16] However, unlike the other chemokines whose function is dependent on CXCR2, DEK requires Gαi protein coupled signaling to mediate its effect on hematopoiesis.[16,43,47] CXCR4 requires Gαi protein-coupled signaling for HSC/HPC migration towards SDF1α as well as providing SDF1α pro-survival signals to myeloid progenitor cells in colony assays.[43] LSK cell migration towards DEK is CXCR2- and Gαi protein-coupled signaling-dependent. It is possible that DEK induces HSC/HPC migration because it functions through a Gαi protein-coupled mediated mechanism like SDF1α. However, IL-8 and MIP2 do not induce migration of HSC/HPC, possibly because their hematopoietic function does not require Gαi protein-coupled signaling for these immature hematopoietic cell populations. This matter requires further investigation.

DEK competes with SDF1α as a chemoattractant agent for LSK cells (Tables 3&4). From these assays it was clear that rmDEK was a more potent chemoattractant for mouse BM LSK cells than rhSDF1α. Interestingly in our previous publication[16], we reported that *in vivo* treatment with rmDEK resulted in a temporary decrease in CXCR4 (the receptor for SDF1α) expression in LSK, LK and HSC populations, which resulted in decreased homing of these cell populations to the BM following an 18-hour homing assay in lethally irradiated mice. The mechanism of

how DEK alters CXCR4 expression on HSC/HPC populations remains unknown. In the case of HSC, HPC, and neutrophils the SDF1α:CXCR4 axis is an important retention signal for these cells to remain in the BM.[23] However, multiple inflammatory signals can disrupt this axis. For example, neutrophil egress from the BM is induced by inflammatory stress conditions (e.g., infection and tissue damage) relying on keratinocyte-derived chemokine (KC), MIP2, IL-8 or the GRO proteins:CXCR2 signaling.[23,39,48,49] *Cxcr2*-deficient mice selectively retain neutrophils in the BM and exhibit neutropenia in circulation.[23,40] Is it possible that DEK secretion, which is induced under inflammatory conditions[2,9,11], might disrupt the retention signal for HSC, HPC and/or neutrophils? Our data suggest that DEK might be involved as a possible compensatory chemotactic agent for HSCs and HPCs under stress/inflammation when SDF1α signaling is reduced.

Authorship

MLC, YS, JR, NM, ML, and DMM conceived the research, designed and performed experiments, interpreted data, and wrote the manuscript.

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Disclosures

N M-V and DMM are co-inventors on a patent for an aptamer that blocks DEK function. All other authors (MLC, YS, JR, ML, HEB) have nothing to disclose.

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Figure Legends

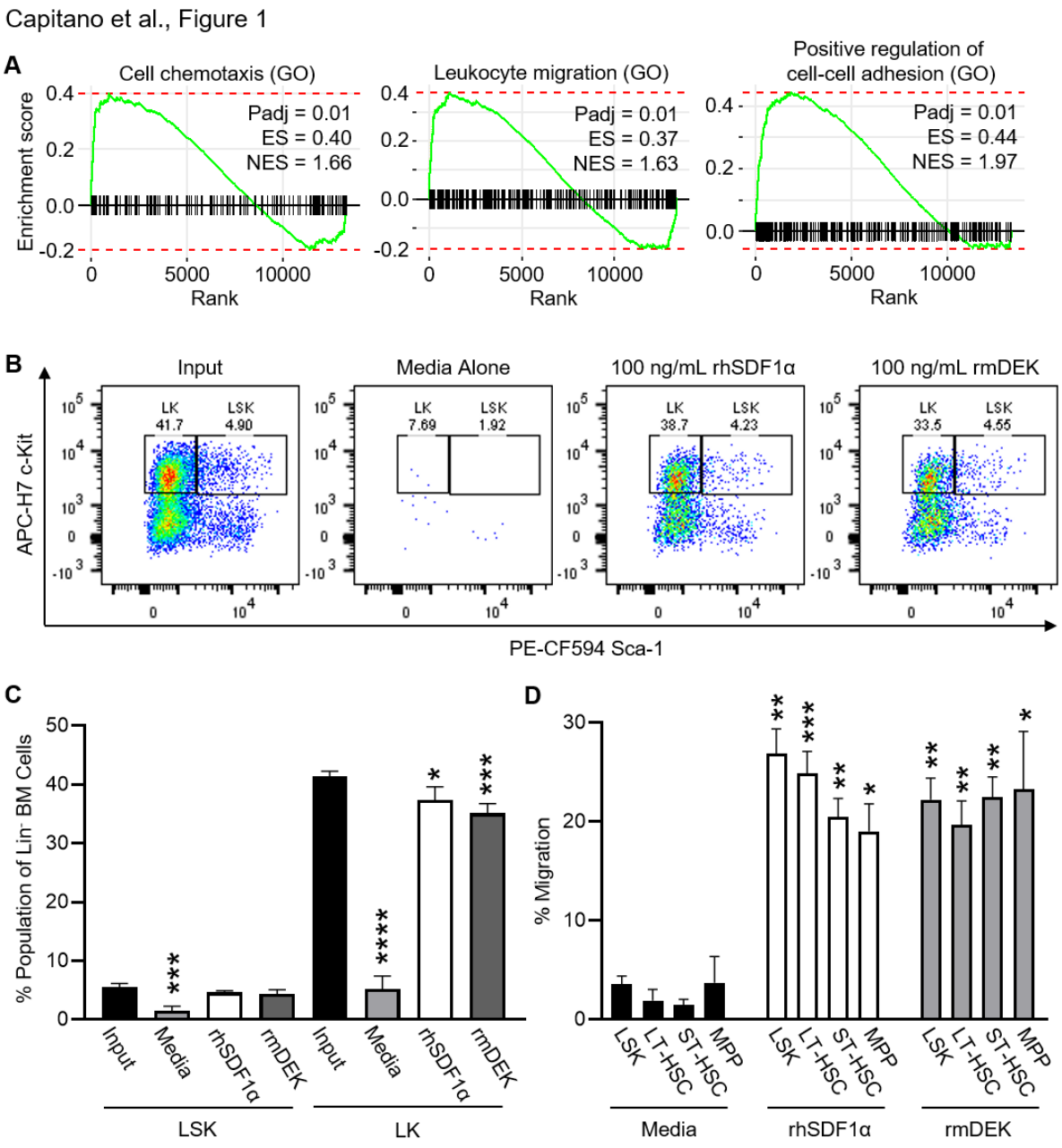

Figure 1. Long-term (LT) hematopoietic stem cell (HSC), short-term (ST)-HSC, and multipotent progenitors (MPP) migrate towards rmDEK. (**A**) Previously generated RNA-seq data (GSE126875) was re-analyzed for differential gene expression and fast gene set enrichment analysis (FGSEA) was performed using publicly available gene sets from MSigDB. Padj= adjusted p-value; ES= enrichment score; NES= normalized enrichment score. (**B**) Representative flow cytometry analysis of input (Lin^- cells) and output samples (Lin^- cells migrating towards wells

containing media alone, 100 ng/mL recombinant human stromal cell-derived factor 1 alpha [rhSDF1α], or 100 ng/mL rmDEK) from a 4 hour transwell migration assay at 37°C. Plots are of Lin^- gated cells. (**C**) Average percent LSK and myeloid progenitor-enriched Lin^- $Sca\text{-}1^-$ $c\text{-}Kit^+$ (LK) cells in the input Lin^- populations and the migrated cell populations from wells containing media alone, 100 ng/mL rhSDF1α, or 100 ng/mL rmDEK. Data represents mean ± SD of 3 replicate wells. Data is a representative of 1 of 3 separate experiments. * $p<0.05$, *** $p<0.001$ and *** $p<0.0001$ when compared to percent input population. (**D**) Migration of LSK cells, LT-HSC (LSK $CD150^+$ $Flt3^-$), ST-HSC (LSK $CD150^+$ $Flt3^+$) and MPP (LSK $CD150^-$ $Flt3^+$) cells towards media alone, 100 ng/mL rhSDF1α and 100 ng/mL rmDEK. Data represents mean ± SD of 3 experiments pooled together. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ when compared to media alone group. (**C-D**) Statistical significance was determined using one-way ANOVA with post-hoc Tukey's multiple-comparisons test using GraphPad Prism 5.0 software.

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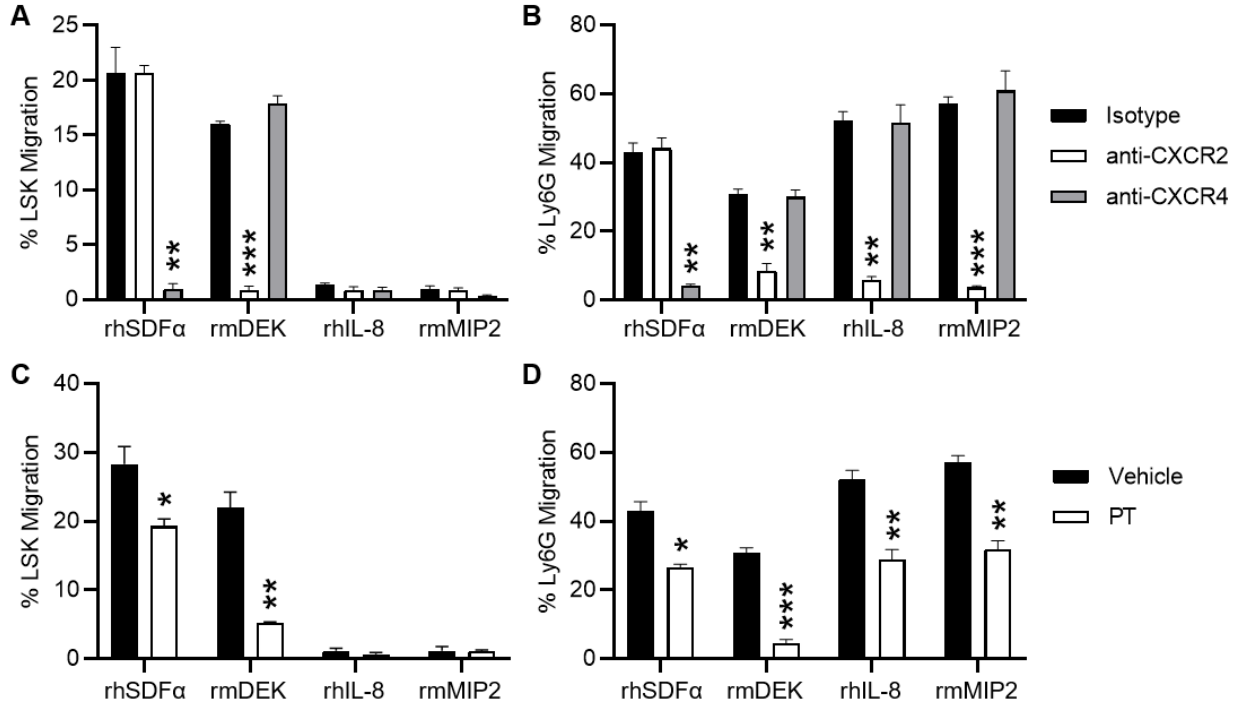

Figure 2. DEK induced migration of LSK and Ly6G$^+$ cells is dependent on CXCR2 and Gαi protein-coupled signaling. (**A-B**) BM Lin$^-$ or Ly6G$^+$ cells were treated with anti-rat IgG (isotype), anti-CXCR2 or anti-CXCR4 neutralizing antibody prior to being placed in the top chamber of a transwell plate and allowed to migrate towards 100 ng/mL rhSDF1α, rmDEK, rhIL-8 or rmMIP2 for 4 hours at 37°C. Total LSK (**A**) or Ly6G$^+$ (**B**) cell migration was determined using flow cytometry with background migration subtracted from total migrated cells. (**C-D**) BM Lin$^-$ or Ly6G$^+$ cells were treated with 1000 ng/mL Pertussis toxin (PT) for 4 hours at 37°C prior to being placed in the top chamber of a transwell plate and allowed to migrate towards 100 ng/mL rhSDF1α, rmDEK, rhIL-8 or rmMIP2 for 4 hours at 37°C. Total LSK (**C**) or Ly6G$^+$ (**D**) cell migration was determined using flow cytometry with background migration subtracted from total migrated cells. (**A-D**) Data are the mean ± SD of triplicate wells. Data are representative of 1 of 3 separate experiments. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ when compared to control for the given chemokine/recombinant protein.

Table 1. Effects of rmDEK on migration of LSK BM cells as assessed by checkerboard assay (see Fig. S2A for experimental design).[a]

rmDEK Concentration (ng/mL) in Lower Chamber	rmDEK Concentration (ng/mL) in Upper Chamber		
	0	50	100
0	0.55% ± 0.09	0.55% ± 0.15	0.65% ± 0.07
50	20.3% ± 0.83	3.82% ± 0.89[b]	2.46% ± 0.35[b]
100	24.4% ± 3.41	6.79% ± 0.69[c]	5.51%± 0.71[c]

[a], Data represents percent migrating LSK BM cells.

[b], $p<0.01$ when compared to group that had 0 ng/mL rmDEK in top chamber and 50 ng/mL rmDEK in bottom chamber.

[c], $p<0.01$ when compared to group that had 0 ng/mL rmDEK in top chamber and 100 ng/mL rmDEK in bottom chamber.

Table 2. Effects of rhSDFα on migration of LSK BM cells as assessed by checkerboard assay (see Fig. S2B for experimental design).[a]

rhSDF1α Concentration (ng/mL) in Lower Chamber	rhSDF1α Concentration (ng/mL) in Upper Chamber		
	0	50	100
0	0.61% ± 0.02	0.35% ± 0.06	0.31% ± 0.06
50	25.4% ± 1.94	2.75% ± 1.26[b]	2.00% ± 0.19[b]
100	27.0% ± 1.38	8.30% ± 0.04[c]	3.16% ± 1.09[c]

[a], Data represents percent migrating LSK BM cells.

[b], p<0.01 when compared to group that had 0 ng/mL rhSDF1α in top chamber and 50 ng/mL rhSDF1α in bottom chamber.

[c], p<0.01 when compared to group that had 0 ng/mL rhSDF1α in top chamber and 100 ng/mL rhSDF1α in bottom chamber.

Table 3. Checkerboard assay to determine if rmDEK can inhibit the migration of LSK BM Cells towards rhSDF1α (see Fig. S2C for experimental design).[a]

rhSDF1α Concentration (nM) in Lower Chamber	rmDEK Concentration (nM) in Upper Chamber			
	0	2.5	5.0	10
0	0.61% ± 0.11	0.32% ± 0.12	0.48% ± 0.33	0.65% ± 0.12
2.5	2.31% ± 0.66	1.26% ± 0.61	0.89% ± 0.36	0.72% ± 0.55
5.0	11.2% ± 3.25	2.11% ± 0.33[b]	0.94% ± 0.47[b]	1.06% ± 0.42[b]
10	23.1% ± 4.86	3.12% ± 1.22[c]	2.49% ± 0.12[c]	1.88% ± 0.50[c]

[a], Data represents percent migrating LSK BM cells.

[b], p<0.01 when compared to group that had 0 nM rmDEK in top chamber and 5.0 nM rhSDF1α in bottom chamber.

[c], p<0.01 when compared to group that had 0 nM rmDEK in top chamber and 10 nM rhSDF1α in bottom chamber.

Table 4. Checkerboard assay to determine if rhSDF1α can inhibit the migration of LSK BM Cells towards rmDEK (see Fig. S2D for experimental design).[a]

rmDEK Concentration (nM) in Lower Chamber	rhSDF1α Concentration (nM) in Upper Chamber			
	0	2.5	5.0	10.0
0	0.53% ± 0.23	0.49% ± 0.10	0.36% ± 0.09	0.86% ± 0.66
2.5	19.8% ± 2.45	14.7% ± 1.28[b]	9.86% ± 3.67[b]	1.36% ± 0.64[b]
5.0	21.3% ± 3.48	15.6% ± 2.39[c]	10.1% ± 2.81[c]	3.32% ± 0.44[c]
10.0	18.6% ± 2.71	20.1% ± 3.45	19.4% ± 6.12	8.44% ± 2.66[d]

[a], Data represents percent migrating LSK BM cells.

[b], p<0.05 when compared to group that had 0 nM rhSDF1α in top chamber and 2.5 nM rmDEK in bottom chamber.

[c], p<0.05 when compared to group that had 0 nM rhSDF1α in top chamber and 5.0 nM rmDEK in bottom chamber.

[d], p<0.01 when compared to group that had 0 nMd rhSDF1α in top chamber and 10 nM rmDEK in bottom chamber.

rmDEK Concentration (ng/mL) in Lower Chamber	rmDEK Concentration (ng/mL) in Upper Chamber		
	0	50	100
0	0.55% ± 0.09	0.55% ± 0.15	0.65% ± 0.07
50	20.3% ± 0.83	3.82% ± 0.89[b]	2.46% ± 0.35[b]
100	24.4% ± 3.41	6.79% ± 0.69[c]	5.51%± 0.71[c]

rhSDF1α Concentration (ng/mL) in Lower Chamber	rhSDF1α Concentration (ng/mL) in Upper Chamber		
	0	50	100
0	0.61% ± 0.02	0.35% ± 0.06	0.31% ± 0.06
50	25.4% ± 1.94	2.75% ± 1.26[b]	2.00% ± 0.19[b]
100	27.0% ± 1.38	8.30% ± 0.04[c]	3.16% ± 1.09[c]

rhSDF1α Concentration (nM) in Lower Chamber	rmDEK Concentration (nM) in Upper Chamber			
	0	2.5	5.0	10
0	0.61% ± 0.11	0.32% ± 0.12	0.48% ± 0.33	0.65% ± 0.12
2.5	2.31% ± 0.66	1.26% ± 0.61	0.89% ± 0.36	0.72% ± 0.55
5.0	11.2% ± 3.25	2.11% ± 0.33[b]	0.94% ± 0.47[b]	1.06% ± 0.42[b]
10	23.1% ± 4.86	3.12% ± 1.22[c]	2.49% ± 0.12[c]	1.88% ± 0.50[c]

rmDEK Concentration (nM) in Lower Chamber	rhSDF1α Concentration (nM) in Upper Chamber			
	0	2.5	5.0	10.0
0	0.53% ± 0.23	0.49% ± 0.10	0.36% ± 0.09	0.86% ± 0.66
2.5	19.8% ± 2.45	14.7% ± 1.28[b]	9.86% ± 3.67[b]	1.36% ± 0.64[b]
5.0	21.3% ± 3.48	15.6% ± 2.39[c]	10.1% ± 2.81[c]	3.32% ± 0.44[c]
10.0	18.6% ± 2.71	20.1% ± 3.45	19.4% ± 6.12	8.44% ± 2.66[d]