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BRIEF CONCLUSIVE REPORT

DEK, a nuclear protein, is chemotactic for hematopoietic stem/progenitor cells acting through CXCR2 and Gαi signaling

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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-1^+ c-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 toward 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.

KEYWORDS
chemokines, chemotaxis, CXCR2, DEK, Gαi signaling, hematopoietic stem and progenitor cells

1 | INTRODUCTION

Nuclear protein DEK, a nonhistone 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, autoantibodies 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 NK cells.[9,11]

Both endogenous DEK and extracellular, recombinant DEK regulate hematopoiesis.[2,9,13–16] Endogenous, nuclear DEK is required for the

Abbreviations: AKT, protein kinase B; BM, bone marrow; C/EBPα, CCAAT enhancer binding protein α; CK2, casein kinase 2; EKLF, erythroid Kruppel-like factor; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; IUSM, Indiana University School of Medicine; Lin^-, lineage negative; LSK cells, Lin^- Sca-1^+ c-Kit^+ cells; LT, long-term; MIP2, macrophage inflammatory protein 2; MPP, multipotent progenitor cells; PT, Pertussis toxin; rh, recombinant human; rm, recombinant mouse; SDF1α, stromal cell-derived factor 1α; ST, short-term.

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 hematopoietic stem cell (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 hematopoietic progenitor cell (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 MAPK.[16] As 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.

2 | MATERIALS AND METHODS

2.1 | 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-h light/12-h 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.

2.2 | RNA-seq analysis

Raw RNA-sequencing reads have previously been deposited in the Gene Expression Omnibus 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$^-$ bone marrow (BM) treated with recombinant mouse (rm)DEK compared with 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 were 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]

2.3 | 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. Prewarmed serum-free IMDM medium (650 μl) 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% 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 h 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 h 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 min at room temperature prior to use and cells washed.

2.4 | 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.

TABLE 1 Effects of rmDEK on migration of LSK BM cells as assessed by checkerboard assay (see Figure S2(A) for experimental design)[a]

	rmDEK concentration (ng/ml) in upper chamber		
rmDEK concentration (ng/ml) in lower 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 represent percent migrating LSK BM cells.
[b]$p < 0.01$ when compared with group that had 0 ng/ml rmDEK in top chamber and 50 ng/ml rmDEK in bottom chamber.
[c]$p < 0.01$ when compared with group that had 0 ng/ml rmDEK in top chamber and 100 ng/ml rmDEK in bottom chamber.

3 | RESULTS/DISCUSSION

3.1 | HSC/HPC-enriched LSK cells migrate toward rmDEK in a time- and dose-dependent manner

To examine novel functions of rmDEK in HSC and HPC regulation, we reexamined 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 with vehicle control-treated cells revealed that gene programs associated with chemotaxis are significantly up-regulated upon treatment with rmDEK (Figure 1(A)). 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 NK 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 toward 100 ng/ml rmDEK with maximum percent migration reached by 4 h in culture (Figure S1(A)). LSK cells migrated toward 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 S1(B)) and 8 h (Figure S1(C)) incubation.

3.2 | rmDEK induces chemotactic, not chemokinetic, movement of LSK cells

Not all factors that influence cellular migration do so in a directional, nonrandom, way (e.g., stem cell factor is chemokinetic for HPC).[24] To determine if DEK mediates chemotactic (directional migration toward a chemoattractant gradient) or chemokinetic (random migration) movement of LSK cells, we performed a checkerboard analysis of LSK cell migration (Figures S2(A) and S2(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 (Figure S2(A)) 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 toward 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 toward SDF1α is chemotactic (Figure S2(B) and Table 2).

3.3 | 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 toward rmDEK and rhSDF1α, the latter as a positive control. LSK and LK cell populations migrated toward rhSDF1α and rmDEK (Figures 1(B) and 1(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 toward rmDEK and rhSDF1α (Figure 1(D)).

3.4 | DEK is a more potent chemoattractant for LSK cells than is SDF1α

As 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 (Figure S2(C) and Table 3) or vice versa (Figure S2(D) 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

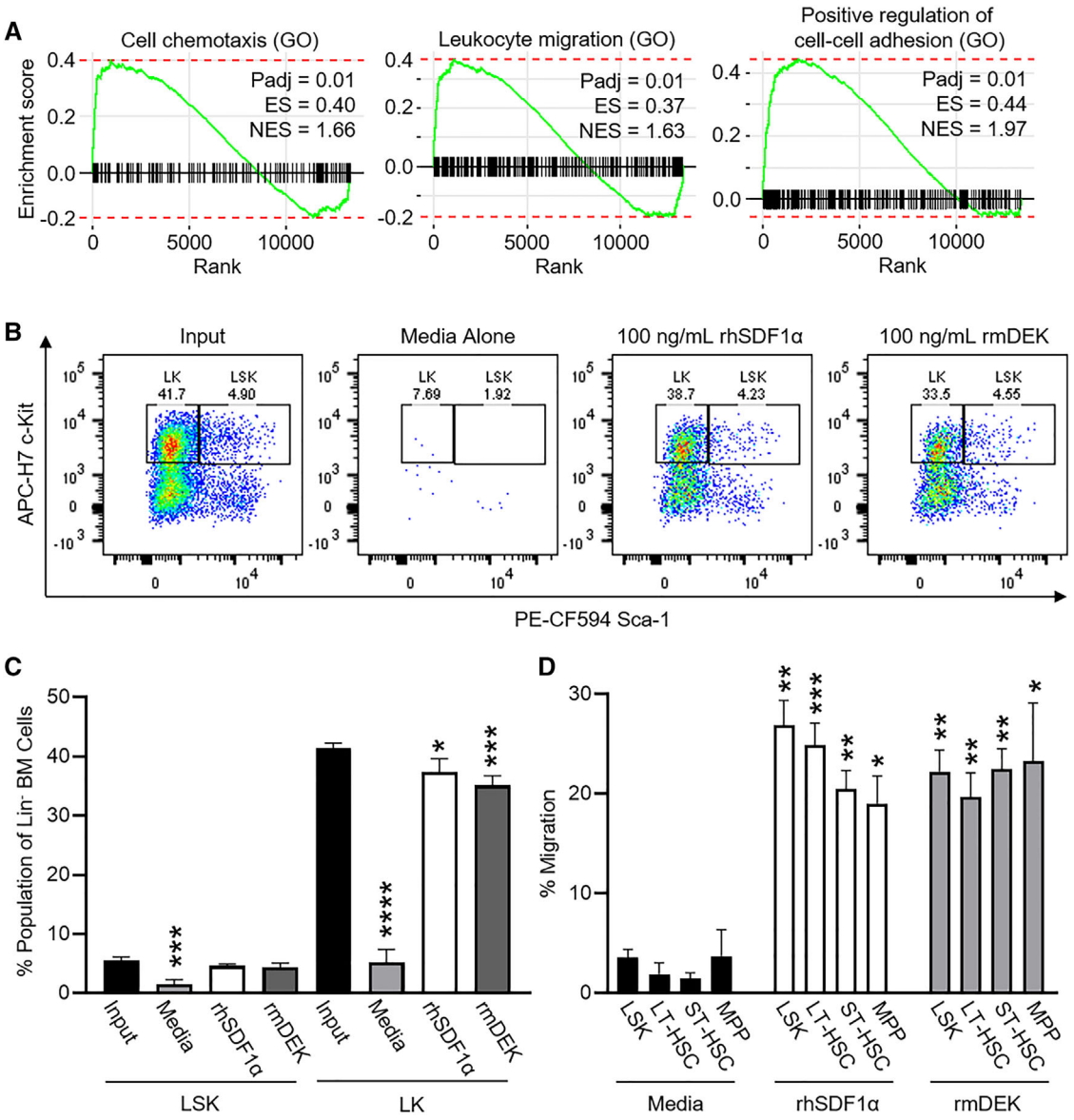

FIGURE 1 Long-term (LT) hematopoietic stem cell (HSC), short-term (ST)-HSC, and multipotent progenitors (MPP) migrate toward rmDEK. (A) Previously generated RNA-seq data (GSE126875) were reanalyzed for differential gene expression and fast gene set enrichment analysis (FGSEA) was performed using publicly 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 toward wells containing media alone, 100 ng/ml recombinant human stromal cell-derived factor 1 alpha [rhSDF1α], or 100 ng/ml rmDEK) from a 4 h 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 represent mean ± SD of 3 replicate wells. Data are representative of 1 of 3 separate experiments. $^*p < 0.05$, $^{***}p < 0.001$, and $^{***}p < 0.0001$ when compared with percent input population. (D) Migration of LSK cells, LT-HSC (LSK $CD150^+$ $Flt3^-$), ST-HSC (LSK $CD150^+$ $Flt3^+$), and MPP (LSK $CD150^-$ $Flt3^+$) cells toward media alone, 100 ng/ml rhSDF1α and 100 ng/ml rmDEK. Data represent mean ± SD of 3 experiments pooled together. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ when compared with media alone group. (C and D) Statistical significance was determined using one-way ANOVA with post-hoc Tukey's multiple-comparisons test using GraphPad Prism 5.0 software

in significant inhibition of LSK cell migration toward 10 nM SDF1α (Figure S2(C) and Table 3). However, it took 10 nM SDF1α to significantly inhibit LSK cell migration toward 10 nM rmDEK (Figure S2(D) and Table 4) suggesting DEK is a more potent chemoattractant for LSK cells.

3.5 | LSK cell migration toward 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

TABLE 2 Effects of rhSDFα on migration of LSK BM cells as assessed by checkerboard assay (see Figure S2(B) for experimental design)[a]

	rhSDF1α concentration (ng/ml) in upper chamber		
rhSDF1α concentration (ng/ml) in lower 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 represent percent migrating LSK BM cells.
[b]$p < 0.01$ when compared with group that had 0 ng/ml rhSDF1α in top chamber and 50 ng/ml rhSDF1α in bottom chamber.
[c]$p < 0.01$ when compared with 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 toward rhSDF1α (see Figure S2(C) for experimental design)[a]

	rmDEK concentration (nM) in upper chamber			
rhSDF1α concentration (nM) in lower 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 represent percent migrating LSK BM cells.
[b]$p < 0.01$ when compared with group that had 0 nM rmDEK in top chamber and 5.0 nM rhSDF1α in bottom chamber.
[c]$p < 0.01$ when compared with 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 toward rmDEK (see Figure S2(D) for experimental design)[a]

	rhSDF1α concentration (nM) in upper chamber			
rmDEK concentration (nM) in lower 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 represent percent migrating LSK BM cells.
[b]$p < 0.05$ when compared with group that had 0 nM rhSDF1α in top chamber and 2.5 nM rmDEK in bottom chamber.
[c]$p < 0.05$ when compared with group that had 0 nM rhSDF1α in top chamber and 5.0 nM rmDEK in bottom chamber.
[d]$p < 0.01$ when compared with group that had 0 nM rhSDF1α in top chamber and 10 nM rmDEK in bottom chamber.

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 (Figure 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 toward 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 toward CXCR4, migration toward DEK was not blocked (Figure 2(A)). To confirm that the neutralizing CXCR2 antibody did not inhibit migration in a nonspecific manner, transwell assays were performed examining LSK cell migration toward rhSDF1α, rhIL-8, and rmMIP2. LSK cells were still able to migrate toward 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 toward rmDEK, rhIL-8, and rmMIP2 (Figure 2(B)). Neutralizing CXCR4 only blocked Ly6G$^+$ neutrophil migration toward rhSDF1α. Chemokine receptors couple to G proteins for signal transduction and this interaction can be blocked using PT, which prevents Gαi proteins

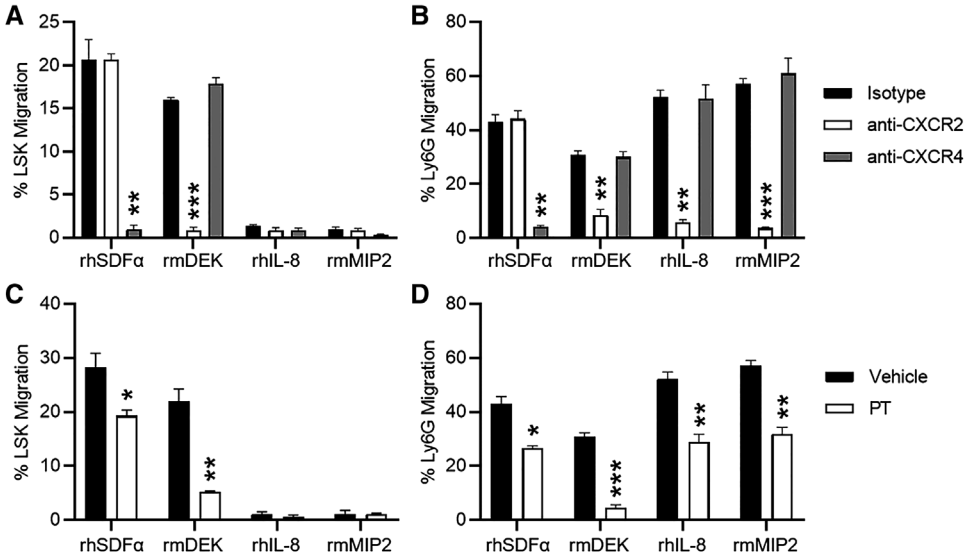

FIGURE 2 DEK-induced migration of LSK and Ly6G$^+$ cells is dependent on CXCR2 and Gαi protein-coupled signaling. (A and B) BM Lin$^-$ or Ly6G$^+$ cells were treated with anti-rat IgG (isotype), anti-CXCR2, or anti-CXCR4 neutralizing antibody prior to being placed in the top chamber of a transwell plate and allowed to migrate toward 100 ng/ml rhSDF1α, rmDEK, rhIL-8, or rmMIP2 for 4 h 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 and D) BM Lin$^-$ or Ly6G$^+$ cells were treated with 1000 ng/ml Pertussis toxin (PT) for 4 h at 37°C prior to being placed in the top chamber of a transwell plate and allowed to migrate toward 100 ng/ml rhSDF1α, rmDEK, rhIL-8, or rmMIP2 for 4 h 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 with control for the given chemokine/recombinant protein

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 toward rmDEK and rhSDF1α (Figure 2(C)). Pretreatment of Ly6G$^+$ neutrophils with PT resulted in significant reduction in migration toward rhSDF1α, rmDEK, rhIL-8, and rmMIP2 (Figure 2(D)). 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 toward SDF1α as well as providing SDF1α prosurvival signals to myeloid progenitor cells in colony assays.[43] LSK cell migration toward 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 and 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-h 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, 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

M. L. C., Y. S., J. R., N. M., M. L., and D. M. M. conceived the research, designed and performed experiments, interpreted data, and wrote the manuscript.

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DISCLOSURES

N. M.-V. and D. M. M. are coinventors on a patent for an aptamer that blocks DEK function. All other authors (M. L. C., Y. S., J. R., M. L., and H. E. B.) have nothing to disclose.

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SUPPORTING INFORMATION

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