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Concise Review: Role of DEK in Stem/Progenitor Cell Biology

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ABSTRACT

Understanding the factors that regulate hematopoiesis opens up the possibility of modifying these factors and their actions for clinical benefit. DEK, a non-histone nuclear phosphoprotein initially identified as a putative proto-oncogene, has recently been linked to regulate hematopoiesis. DEK has myelosuppressive activity in vitro on proliferation of human and mouse hematopoietic progenitor cells and enhancing activity on engraftment of long-term marrow repopulating mouse stem cells, has been linked in coordinate regulation with the transcription factor C/EBPα, for differentiation of myeloid cells, and apparently targets a long-term repopulating hematopoietic stem cell for leukemic transformation. This review covers the uniqueness of DEK, what is known about how it now functions as a nuclear protein and also as a secreted molecule that can act in paracrine fashion, and how it may be regulated in part by dipeptidylpeptidase 4, an enzyme known to truncate and modify a number of proteins involved in activities on hematopoietic cells. Examples are provided of possible future areas of investigation needed to better understand how DEK may be regulated and function as a regulator of hematopoiesis, information possibly translatable to other normal and diseased immature cell systems. STEM CELLS *2013;31:1447–1453*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Hematopoietic stem cell (HSCs) and hematopoietic progenitor cells (HPCs) give rise to all blood forming elements and have been used to successfully treat non-malignant and malignant disorders [1, 2]. However, much remains to be deciphered regarding regulation of HSC and HPC function and fate. In efforts to uncover key factors involved in HSC and HPC production and fate decisions, we identified DEK, a biochemically distinct mammalian nuclear phosphoprotein initially classified as a putative proto-oncoprotein [3], as a candidate for regulating hematopoiesis [4]. We noted that DEK had negative regulatory effects on proliferation of HPCs: granulocyte–macrophage (CFU-GM), erythroid (BFU-E), and multipotential (CFU-GEMM), but positive effects on engrafting HSCs [4]. Others have linked DEK in coordinate regulation with the transcription factor C/EBPα on the differentiation of myeloid cells [5]. This complex of C/EBPα and DEK, whose assembly and disassembly is regulated by serine 21 phosphorylation of C/EBPα, enhanced the activation of the granulocyte-colony stimulating factor receptor 3 promotor. Knocking down expression of the *DEK* gene reduced the capacity of C/EBPα to drive granulocyte target gene expression. We recently reported that the cell surface enzyme CD26, a dipeptidylpeptidase 4 (DPP4), truncates and changes the functional activities of cytokines such as the colony stimulating factors (CSFs: GM-CSF, granulocyte-CSF [G-CSF], interleukin-3 [IL-3], and erythropoietin [EPO]) and of the chemokine, stromal derived factor-1 (SDF-1/CXCL12) [6, 7]. We now know that other proteins with cell regulatory activity have putative truncation sites for DPP4 [8, 9]. As discussed below, DEK has a putative specific truncation site for DPP4. Based on this information, we hypothesize that DEK is a key and perhaps crucial regulatory determinant of HSC and HPC function and fate decisions in both steady-state and stressed hematopoiesis, effects that may be mediated or regulated by DPP4 truncation of DEK.

There is still a paucity of information on DEK and its actions. This review covers current knowledge of DEK and its role in cell regulation and fate decisions, with a particular emphasis on HSC, HPC, and hematopoiesis. Examples are suggested for future studies in this area.

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DEK Regulation and Activities

DEK bears little resemblance to other known proteins (Fig. 1A), and is the only representative of its own protein class. While DEK, an abundant non-histone chromosomal factor, is vital to global heterochromatin integrity [10] (Fig. 1B, 1C), it can be secreted by certain cells, sometimes in exosomes, or in its free form and subsequently be taken up as the same molecule in bioactive form in a heparin sulfate-dependent process by other cells where it, in turn, modulates global chromatin structure [11], a process similar to what is seen in a paracrine loop. Whether uptake of DEK can also take place through G-Protein-coupled receptors or whether DEK can act through stimulation of such receptors is not clear, and this may vary between cell types and their maturational status.

DEK is heavily post-translationally modified. Regulation of the phosphorylation status of DEK by CK2 and protein phosphatase 2A [12, 13], acetylation [14, 15], and poly (ADP-ribosyl)ation [16, 17] can regulate the function of DEK. Transcription of the *DEK* gene is controlled by YY1 and NF-Y [18], E2F [19], and the estrogen receptor α [20]. The DEK protein can be degraded by the F-box/WD repeat containing protein 7 (Fbxwt) [21], and microRNA-489, which is involved in the maintenance of muscle stem-cell quiescence, does so by targeting *DEK* [22]. DEK interacts with RelA/p65 [23] and represses gene expression in conjunction with SET and PARP1 [24]. Phosphorylation by protein kinase CK2 [12] in one way affects the function of the DNA-binding domains (SAP-box and C-terminal DNA binding domain) of DEK [25] and in another way allows for the histone chaperone functions of DEK that co-activate transcription of a nuclear receptor [26]. However, CK2 does not seem to play a role in regulating DEK's function in chromatin integrity [12].

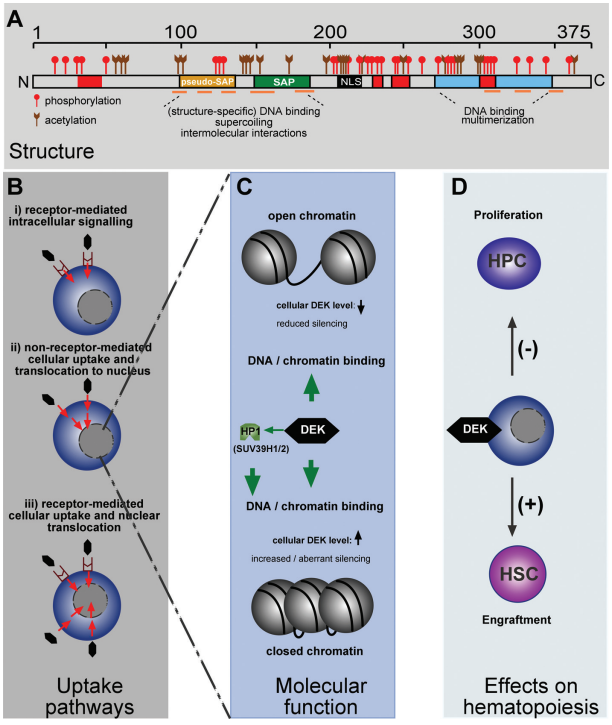

Figure 1. Schematic depiction of the structure, functional domains, and post-translational modifications of DEK, potential DEK uptake pathways, and effects of DEK on hematopoiesis. **(A):** Linear sequence of DEK with DNA binding domains (yellow: pseudo-SAP-box; green: SAP-box; blue: C-terminal DNA-binding domain; orange lines: position of α-helices, as revealed by nuclear magnetic resonance), and other functional features (red: acidic regions; black: putative NLS) indicated [See refs. 25, 41, 42, 44, 45]. The positions of previously mapped phosphorylation and acetylation sites are marked [See refs. 12, 14, 15]. **(B):** Potential DEK receptor mediated and/or uptake pathways. **(C):** Simplified depiction of DEK functions in the setting of chromatin. DEK interacts with, and augments binding of HP1 to H3K9Me3, thereby recruiting SUV39H1/2, thus further enhancing the deposition of H3K9Me3. In addition, DEK binds to DNA/chromatin via its DNA binding domains (see **A**). However, the precise consequences for open or closed chromatin currently remain elusive. **(D):** Functional consequences of DEK in the setting of hematopoietic stem and progenitor cells. Not shown is the potential influence of dipeptidylpeptidase 4 truncation of DEK on DEK functional activity. Abbreviations: HPC, hematopoietic progenitor cells; HSC, hematopoietic stem cells.

Blood cell production is regulated by cytokine and microenvironmental influences that direct HSC and HPC functions and fate decisions [2, 27]. Knowledge of these influences and how they are mechanistically mediated is crucial to understanding hematopoiesis under steady-state and stress conditions, and eventually for correction of the abnormalities in hematopoiesis associated with disease, and for optimal efficacy in the use of HSC and HPC for hematopoietic cell transplantation. Our knowledge of the extracellular and intracellular factors influencing the proliferation, self-renewal, survival, differentiation, and movement of HSC and HPC is increasing [2, 27], but there is still much to be learned if we are to best use this information for improved health care. In continuing efforts to define new factors in the regulation of hematopoiesis, we focused on DEK, a non-histone phosphoprotein which was initially identified as a fusion protein resulting from a t(6;9) translocation in a rare subtype of acute myelogenous leukemia [3]. Furthermore, DEK is overexpressed and implicated in many malignancies [3, 28–38] and exhibits critical functions in several central tumor-promoting pathways, for example, inhibition of apoptosis and senescence, among others [3, 28, 30, 33, 37, 39, 40]. DEK bears little resemblance to other known proteins, but is well-conserved among higher eukaryotes, as all DEK proteins share a unique conserved region, the "SAP-box" (SAP = Saf/Actinus/PARP) [41]. This motif is found in proteins such as DEK that are typically involved in DNA binding, chromatin remodeling, and/or RNA processing [41–43]. DEK is capable of binding to the TG-rich *pets* site in the human immunodeficiency virus type 2 (HIV-2) promoter, where it acts as a transcriptional repressor [13, 44], although it appears that DEK primarily recognizes DNA on the basis of structure rather than sequence and thus might play an active role in maintaining higher-order chromatin architecture [42, 43, 45, 46]. In addition to its DNA binding properties, DEK is found in association with messenger RNA splicing and export factors, as well as with spliced transcripts, where it influences 3′ splice fidelity [46–50]. Intense post-translational modification of DEK by phosphorylation [12], acetylation [14–16], and poly(-ADP-ribosyl)ation [17] point to the importance of these post-translational modifications in regulating DEK's multiple functions and subcellular or extracellular localization. DEK antibodies are found in patients with juvenile idiopathic arthritis and other auto-immune diseases [15, 51, 52], raising the question of why this nuclear protein is an autoantigen. Although DEK is primarily associated with chromatin throughout the cell cycle [53], two independent pathways, both involving post-translational modifications, were recently identified that result in DEK's presence in the extracellular space. The first pathway implicated nonclassical secretion of DEK by activated human monocyte-derived macrophages in both a free form and in exosomes [54]. In the second, passive release of

poly(ADP-ribosyl)ated, hyperphosphorylated DEK by apoptotic T-lymphocytes was observed possibly occurring as a result of Fas-ligand- or stress-mediated apoptosis [16]. IL-8 induces the secretion of DEK, and DEK acts as a chemoattractant for peripheral blood leukocytes [54]. Of particular note in this context, secreted DEK can be taken up by other cells, move to the nucleus, and effectively carry out the intranuclear functions of DEK, including control of global heterochromatin integrity and DNA repair [11].

DEK and Hematopoiesis

Extracellular and intracellular DEK expression modulates hematopoiesis [4]. It remains to be determined exactly how DEK is mediating its different effects on hematopoiesis. This may be through receptor mediated cytokine-like activities in which a sequence of intracellular signals are induced, or perhaps by cellular uptake through a specific receptor, and/or non-receptor mediated DEK uptake. Uptake of DEK through receptor-mediated or non-receptor-mediated events may involve chromatin regulation (Fig. 1B, 1C). These different possibilities require additional investigation. Exploring these possibilities is important for understanding the effects of DEK and perhaps for casting new light on regulation of hematopoiesis during health and disease.

We found that DEK, in purified recombinant human form, was myelosuppressive for colony formation by CFU-GM, BFU-E, and CFU-GEMM from C57Bl/6 mouse bone marrow (BM) and human CB cells, effects that were dose-dependent [4]. The suppressive effects of DEK on colony formation were apparent when assayed on single isolated $CD34^+$ cord blood (CB) cells, suggesting direct acting effects of DEK on HPC although how these effects are mediated is not known. This negative regulatory role of DEK was consistent with the enhanced numbers and cycling status of CFU-GM, BFU-E, and CFU-GEMM in the BM and spleen of C57Bl/6 $DEK^{-/-}$, compared with C57Bl/$6^{+/+}$, mice [4]. Some of this effect may reflect the reports of others showing that DEK works in concert with C/EBPα to regulate differentiation of myeloid cells [5]. Most recently, we found similar effects using cells from $DEK^{-/-}$ mice on another mouse strain background. DBA/1 $DEK^{-/-}$ HPCs were at a higher cell cycling rate than DBA/1 $DEK^{+/+}$ HPCs (Fig. 2). Moreover, purified recombinant human DEK-inhibited colony formation of DBA/$1^{+/+}$ CFU-GM, BFU-E, and CFU-GEMM in dose-response fashion (Broxmeyer unpublished observations). These results demonstrate that the findings are not confined to effects on only one mouse strain. In contrast to the inhibition of CFU proliferation by DEK, BM cells from C57Bl/6 $DEK^{-/-}$ mice manifested decreased longer-, but not short-, term competitive repopulation capability in lethally irradiated congenic mice, in addition to even greater decreases in repopulation of lethally irradiated secondary mouse recipients in a noncompetitive assay [4]. This suggested that DEK was important for the positive engrafting capability of a long-term, but not short-term, repopulating and self-renewing HSC (schematically shown in Fig. 1D). The results suggest that DEK may be necessary for maintenance of HSC, consistent with reports that DEK is expressed in immature cell populations and that this expression decreases with the differentiation and maturing of the cells [55]. Interestingly, the DEK/CAN fusion protein resulting from the t(6;9) chromosomal translocation [3] targets a long-term repopulating HSC for leukemic transformation [56].

Still to be determined are effects of exogenously added DEK on HSC and HPC numbers and functional activity, and hematopoiesis in vivo, and possible effects of DEK on ex vivo expansion of HSC and HPC, information that could possibly be of therapeutic use. The functional DEK domains that may be involved in DEK receptor-binding and/or non-receptor mediated uptake and translocation to the nucleus remain to be determined, although DEK does have a putative nuclear localization domain (Fig. 1).

DEK and DPP4

We recently found that in addition to the homing and chemotactic protein SDF-1/CXCL12 [7, 57], GM-CSF, G-CSF, IL-3, and EPO have truncation sites for the enzyme DPP4 [6]. DPP4 is found on the surface of many cells as CD26 and is present within cells expressing CD26. It is also found as a soluble enzyme in serum and plasma. DPP4 treatment of SDF-1/CXCL12 produces a molecule in which the two N-terminal amino acids have been removed. Truncated SDF-1/CXCL12 is inactive as an HPC chemotactic molecule and as an HPC survival enhancing factor, and this truncated SDF-1/CXCL12 can block the chemotactic and survival enhancing

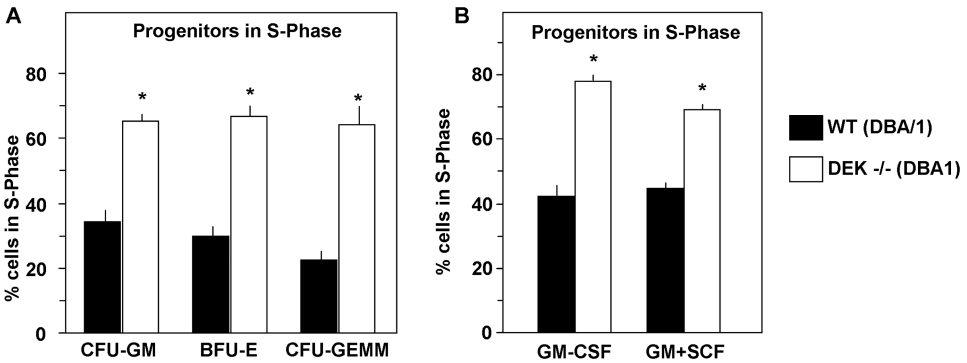

Figure 2. Effects of DEK knockout (−/−) on hematopoietic progenitor cells. Results are shown for these cells in S-phase of the cell cycle, as determined by high specific activity tritiated thymidine kill technique, when stimulated with 5% pokeweed mitogen spleen cell conditioned medium (PWMSCM), SCF (50 ng/ml), and erythropoietin (1 U/ml) in methylcellulose culture **(A)** and when stimulated with GM-CSF (10 ng/ml) alone and in combination with SCF in agar culture **(B)** [6]. Results are given as mean ± SEM of three mice per group. *, significantly different from control medium ($p < .05$), as assessed by two tailed *t*-test. Abbreviations: BFU-E, burst forming unit-erythroid; CFU, colony forming unit; CSF, colony stimulating factor; GEMM, granulocyte–macrophage multipotential; GM, granulocyte–macrophage; SCF, stem cell factor; WT, wild type.

effect of the full-length SDF-1/CXCL12 molecule [6, 7]. DPP4 treatment of GM-CSF, G-CSF, IL-3, and EPO produced truncated forms of these CSFs that had greatly decreased CSF activity, but blocked the activity of the full-length forms of their respective CSF, for colony formation in vitro, and with human GM-CSF for intracellular signaling (phosphorylation) of JAK2 and STAT5 using TF1, a human factor dependent cell line, and primary $CD34^+$ CB cells [6]. These in vitro effects on HPC proliferation were duplicated in vivo in mice given exogenously added full-length, truncated, or the combination of full-length and truncated GM-CSF, and also for full-length and truncated EPO [6]. The effects were most apparent on target cells pretreated with an inhibitor of DPP4 (e.g., Diprotin A; a tripeptide: ILE-PRO-ILE) or in $CD26^{-/-}$ mice. Both radiolabeled full-length and truncated human GM-CSF demonstrated receptor binding to the GM-CSF receptor of TF1 cells and primary $CD34^+$ CB cells, but the truncated GM-CSF bound to the GM-CSF receptor with higher affinity (had a lower dissociation constant), and truncated GM-CSF blocked receptor binding of full-length GM-CSF at concentrations of truncated GM-CSF one-eighth that of full-length GM-CSF [6]. Thus, DPP4-treated and truncated SDF-1/CXCL12, GM-CSF, G-CSF, IL-3, and EPO act as dominant negative or competitive molecules for the actions of their respective full-length proteins, and offer a potential means for regulating specific protein actions, and it may be that DEK works in a similar fashion.

DPP4 can truncate proteins at a penultimate alanine or proline at the N-terminus and also when serine or other amino acids are at this penultimate site [8, 9]. Most recently, we found that the N-terminus start site of the DEK protein contained a putative DPP4 truncation site (MSASAPAAEGEGTPTQP …) in which serine (the second amino acid in this sequence), rather than alanine or proline, served as a potential site for DPP4 activity, unless in this case methionine is preclipped off and it is the alanine (the second amino acid in the following N-terminus sequence) that serves as a DPP4 truncation site (SASAPAAEGEGTPTQP …). As shown in Figure 3, DPP4-treated DEK did not manifest inhibitory activity against GM-CSF- or GM-CSF plus SCF stimulated colony formation of mouse BM cells pretreated with Diprotin A, an inhibitor of DPP4, and at one-fourth to one-eighth the concentration of untreated DEK, the DPP4-treated DEK blocked the inhibitory activity of full-length DEK, suggesting that DPP4-treated DEK can act as a dominant negative effector molecule for full-length DEK, perhaps at the level of specific DEK receptor binding or non-receptor mediated uptake. Whether DPP4 truncates at the second amino acid (serine) or if the methionine is preclipped and DPP4 truncates at the alanine that follows the serine, this would leave the next sets of amino acids open, with potential additional truncation sites (serine, alanine, or proline) available, so that DPP4 may be able to continue to truncate DEK. It remains to be determined by mass spectrometry or other analysis what the effects of DPP4 are on the DEK protein itself and where exactly the DPP4 may be acting. Such analysis needs to be linked to functional effects of DEK. These results, which need to be pursued in greater depth both in vitro and in vivo, suggest a potentially strong and modifying influence of DPP4 on DEK activity, which may be of physiological relevance, and of potential clinical interest.

Future efforts to more precisely define hematopoiesis in $DEK^{-/-}$ mice seem reasonable in the context of steady-state hematopoiesis, and especially for hematopoiesis in mice subjected to different stresses. We recently evaluated the effects of radiation and drugs in $CD26^{-/-}$ mice [6]. Stress situations can help define the relevance of DEK in a way that may not be picked up using untreated mice. The stresses to be evaluated for hematological recovery can be low and higher non-lethal γ-irradiation, and non-lethal doses of drugs such as 5 fluorouracil and cyclophosphamide (Cytoxan).

In depth studies on mechanisms of DEK activities might best be carried out on relevant established cell lines with confirming studies on primary target cells, such as we recently published for GM-CSF [6]. We have identified the human factor-dependent cell lines, TF1 (responsive to stimulation of proliferation by GM-CSF, IL-3, or EPO, and when used in combination with SCF eliciting a synergistic stimulating effect) and M07e (responsive to GM-CSF or IL-3, and synergistically to either GM-CSF or IL-3 with SCF) as responding to the inhibitory effects of DEK with a 1 hour pulse exposure of these cell lines to DEK (Fig. 4). TF1 [6] and

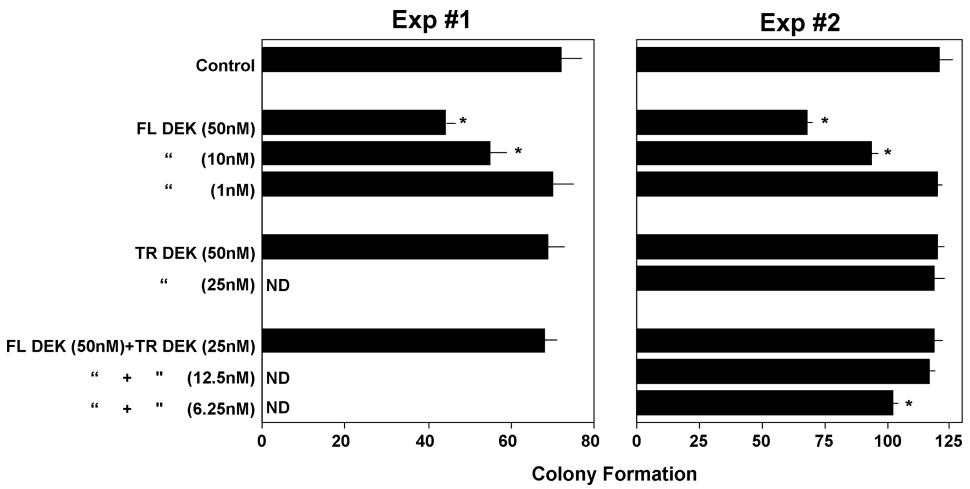

Figure 3. Influence of dipeptidylpeptidase 4 (DPP4) on DEK activity. Shown are effects of full-length (FL) – and DPP4-treated (= likely truncated, TR) DEK, alone and in combination, on 5×10^4 C57Bl/6 mouse BM cells/ml pretreated with diprotin A, an inhibitor of DPP4, and stimulated with GM-CSF (Exp#1) or GM-CSF+SCF (Exp#2) (See ref. 6] for details of such studies carried out with other growth modulating proteins). Results are given as mean ± SEM. *, significantly different from control medium ($p < .05$). Abbreviations: FL, full-length; ND, not done; TR, truncated.

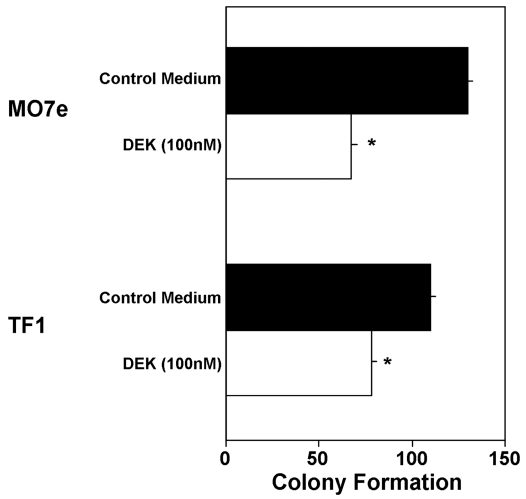

Figure 4. Influence of DEK on colony formation by human factor dependent cell lines MO7e and TF1. These cells were not pretreated with a dipeptidylpeptidase 4 inhibitor. Results are shown as mean ± SEM for 1000 MO7e and 500 TF1 cells/ml ± 1 hour pulse treatment of these cell lines with control medium or 50 nM DEK before their stimulation with GM-CSF plus SCF [See ref. 6] for details of such studies carried out with the TF1 human growth factor dependent cell line assessing the effects of other proteins on colony formation). *, significantly different from control medium ($p < .05$).

M07e cells express active DPP4 on their cell surface as CD26, (H. O'Leary and H.E. Broxmeyer, unpublished studies), as do HPC and HSC in primary mouse and human BM and human CB [6, 7]. Although the effects of cytokines with DPP4 truncation sites are more potent on CD26-expressing cells that have been pretreated to inhibit DPP4 [6] (H.E. Broxmeyer, unpublished observations), the activities of these cytokines can still be detected although at a lesser effect if the CD26-expressing cells are not pretreated to inhibit DPP4. Much work remains to elucidate a role for DPP4 on protein activity [8, 9]. Hence, although the TF1 and M07e cells were not first pretreated to inhibit DPP4, we did detect DEK inhibition of colony formation by these cell lines. As both cell lines have been used by us as models for stimulating and negatively acting cytokines at a cell and intracellular level [58–68], these growth factor dependent human cell lines can be used as models to initiate intracellular studies on DEK signal transduction, effects which can then be verified in less extensive studies using, for example, primary $CD34^+$ CB cells, as we reported previously for GM-CSF [6].

As communication between BM microenvironmental cells and the HSC and HPC that reside in the BM is involved in HSC and HPC function and fate decisions, and DEK is expressed in osteoblasts, endothelial cells, and BM stromal cells (authors of the studies in ref. 69–71 deposited their gene expression data sets into the GEO profiles), DEK may be involved in microenvironmental cell-HSC/HPC interactions, perhaps with regards to positive effects we have noted with DEK on engrafting capability of HSC and DEK negative regulation of HPC. Such possible influences can be investigated in a stem cell transplant model that can distinguish donor (e.g., $CD45.2^+$) from recipient (e.g., $CD45.1^+/CD45.2^+$ F1) from competitor (e.g., $CD45.1^+$) cells in a competitive HSC setting, and also donor from recipient cells in a non-competitive HSC transplant assay. $DEK^{-/-}$ mice (on a C57Bl/6 mouse strain background) can be used alternatively as recipients and/or as a source of donor cells.

Concluding Comments

In summary, DEK is an apparently unique molecule whose functional activity in the normal regulation of HSC and HPC cells [4, 5], and in leukemogenesis [56], is only just beginning to be elucidated. It is likely that DEK will be found to have controlling roles in other stem and progenitor cell types in addition to those in muscle [22], including embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem/stromal cells. Abnormalities in DEK and its actions may be associated with cancer and cancer stem/progenitor cells [28, 72]. How DEK fits in with other chromatin and intracellular molecules involved in regulation of HSC and HPC [2, 27] is of great interest, and remains to be determined. DEK modulates global heterochromatin integrity in human cells and in a *Drosophila* model [10]. Interference with DEK expression in human cells induces a phenotype indicative of more accessible chromatin organization. DEK is a Su(var), meaning that it is a positive modulator of heterochromatin as shown using the model of white-mottled variegation in the eyes of *Drosophila* [10] (Fig. 1C). Loss of DEK is accompanied by loss of the key heterochromatic marker H3K9Me3, and both phosphorylated and unphosphorylated DEK interact with the vital heterochromatin factor HP1α, thus bringing HP1α to the H3K9 mark. DEK also plays a key role in epigenetic silencing by bringing the KMT1 A/B methylase (which adds the third methyl group to H3K9), along with HP1α, to what becomes the H3K9Me3 mark, a hallmark of heterochromatin [10]. This was shown by CHIP analysis, co-immunoprecipitation, and biochemical interactions. Thus, it is of interest to determine if uptake of DEK into HSCs and HPCs leads to changes in chromatin architecture with the subsequent effects on gene expression leading to effects on hematopoiesis. To further understand the mechanism of action of DEK in hematopoiesis, it will be essential to determine whether DEK effects are mediated via specific receptors with subsequent induction of intracellular signaling and/or by uptake and translocation of DEK into the cytoplasm and subsequently into the nucleus (Fig. 1B, 1C). It will be important to identify the domains of DEK that are responsible for the effects on HSC and HPC, and whether they are the same domains that are involved in DNA binding and heterochromatin integrity [23]. A search for a specific receptor or receptors for DEK is currently ongoing. In the context of tumor cell sensitivity to chemotherapy, upregulation of DEK expression may enhance cell survival and chemoresistance, while decreasing DEK expression in such cells may enhance the sensitivity of the tumor cells to kill by specific chemotherapeutic agents [33, 34, 39, 73]. As DEK expression is higher in immature compared with more mature blood cells such as those present in the $CD34^+$cell population [55], and leukemia (LIC) or tumor (TIC) initiating cells present in the $CD34^+$ cell population [2, 27], DEK could be one of the reasons that LIC or TIC populations survive chemotherapy. Also of interest is the effect of DPP4 truncation of DEK on these functional activities of DEK. Therefore, further understanding the function and molecular mechanisms of action of this biochemically distinct protein, including assessment of protein partners, DNA and RNA targets and the secretion and uptake by neighboring cells, is likely to yield information relevant to clinically important questions.

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Disclosure of Potential Conflicts of Interest

H.E.B. is a member of the Medical Scientific Advisory Board of Corduse, a cord blood banking company based in Orlando, Florida. All other authors indicate no potential conflicts of interest.

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