

Transcriptional regulation of 1-cys peroxiredoxin by the proto-oncogene protein DEK

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Abstract. The nuclear phosphoprotein DEK is abundantly present in cells and is implicated in diseases including leukemia and autoimmune disorders. DEK has three highly acidic amino acid domains and inhibits histone acetyltransferase activity by binding directly to histones. In a previous study, differentially regulated proteins under DEK knockdown conditions, including the up-regulated protein 1-cys peroxiredoxin (1-cys Prx), were identified by proteome analysis. Here, an *in vivo* reporter assay with short hairpin RNA-mediated DEK knockdown revealed that DEK negatively regulated 1-cys Prx transcription, and that the NF- κ B subunit p65 had a synergistic effect on this DEK-mediated repression. Both proteins are recruited to the 1-cys Prx promoter region and regulate its transcription. Our study demonstrates that DEK modulates the transcriptional regulation of the target gene through protein interaction with other regulatory proteins.

Introduction

The DEK protein was first identified in a specific chromosomal translocation t(6;9)(p23;q34) in an acute myelogenous leukemia, which results in the formation of a fusion gene with the CAN nucleoporin protein NUP214 (1). This translocated fusion gene makes a fusion protein, in which part of the N-terminal (1-349 amino acids) of DEK is fused with part of the C-terminal (813-2,090 amino acids) of CAN. The DEK protein, as a phosphoprotein, is an abundantly and ubiquitously expressed nuclear protein with two functional domains (SAP and DNA binding/multimerization), several phosphorylation sites and three highly acidic domains (2,3). DEK binds DNA and induces DEK-DEK multimerization in a phosphorylation-dependent manner (4). A recent report

showed that a highly acidic domain in DEK inhibits histone acetyltransferase (HAT) activity through histone binding (2). It has been revealed that DEK influences chromatin remodeling through the alteration of chromatin topology (5,6). DEK also regulates transcriptional activity by recruiting transcriptional co-repressors, such as hDaxx and histone deacetylase-2, to chromatin (7).

DEK is also involved in carcinogenesis and autoimmune diseases, such as systemic lupus erythematosus, juvenile rheumatoid arthritis and *ataxia telangiectasia* (8). A recent proteomic analysis revealed that DEK knockdown up-regulated 1-cys peroxiredoxin (1-cys Prx) (Prx 6) and caused the hyperacetylation of histones around the 1-cys Prx promoter (9).

1-cys Prx is one of six Prx isoforms, and is expressed in various tissues – in particular liver tissues – related to the protection of cellular oxidative stresses (10).

NF- κ B is a transcription factor with multiple biological functions, including immune and inflammatory response, cell growth and differentiation, and the suppression of apoptosis (11). The NF- κ B family consists of five members, p65/RelA, p50 (p105), p52 (p100), RelB and c-Rel (12). A previous study showed that the p65 subunit of NF- κ B interacts with DEK (13).

In this study, we identified the negative regulatory role of DEK in 1-cys Prx transcription: its knockdown rescues the transcriptional activation of 1-cys Prx through the p65 subunit of NF- κ B. Additionally, we showed that the p65 subunit of NF- κ B synergistically represses 1-cys Prx by recruitment to the promoter.

Materials and methods

Plasmid constructs. For the reporter assay, the following pCMX plasmid constructs were used: CMX-DEK (wild-type), CMX-DEK Δ 290 and CMX-DEK Δ 200 (2). The p65 subunit coding region was obtained by PCR amplification using the following specific primers: *Bam*HI site-linked 5'-CGCGGA TCCATGGACGAACTGTTCCCCCTC-3' (forward), and *Xho*I site-linked 5'-CGCCTCGAGGGAGCTGATCTGACTCAG CAG-3' (reverse). Gel-purified PCR product was cloned into the modified pcDNA6-myc vector by *Bam*HI-*Xho*I enzyme digestion, which has a triple-tag sequence (myc, his and HA).

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Knockdown of endogenous DEK. To suppress DEK expression, a vector-based RNAi method was used. pSM2c-DEK expressing DEK specific short hairpin RNAs (nucleotides 654-674 from NM_003472), referred to as shRNA, was purchased from Open Biosystems (Huntsville, AL, USA).

Cell culture and luciferase reporter assay. HeLa cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) containing 10% heat-inactivated fetal bovine serum (Invitrogen) and 0.05% penicillin-streptomycin (Invitrogen) at 37°C in a 5% CO₂ humidified atmosphere. HeLa cells were seeded in 48-well dishes and transfected by lipofectamine 2000 (Invitrogen) with pGL3-1-cys Prx (100 ng) in the absence or presence of pcDNA6-p65-myc (50, 100, 150 or 200 ng) or shDEK (50, 100, 150 or 200 ng), and each of the deletion mutants (wild-type, Δ290 and Δ200; 50, 100, 200 or 300 ng). To demonstrate the role of DEK in p65-mediated transcriptional regulation, pGL3-1-cys Prx (100 ng) was transfected with pcDNA6-p65-myc and further transfected with increasing amounts (50 and 100 ng) of shDEK plasmid. Luciferase activity was measured by the addition of 20 μl luciferin as a substrate into 80 μl of cell lysates using the Glomax luminometer (Promega, Pittsburg, PA, USA). Each value is the mean of four replicates from a single assay. The results are representative of at least three independent experiments.

Chromatin immunoprecipitation analysis. HeLa cells were transfected with CMX-DEK (4.8 μg) or shDEK (3 μg) plasmids and harvested at 48 or 72 h post-transfection. The cells were crosslinked with 1% formaldehyde in medium for 10 min at 37°C followed by the addition of 125 mM glycine for 5 min at room temperature, and were then scraped into SDS lysis buffer. Cell lysates were sonicated and diluted for Chromatin immunoprecipitation (ChIP) with the antibodies: anti-p65 (5 μg), anti-myc (5 μg) and anti-DEK (5 μg) (Santa Cruz Biotechnology, Paso Robles, CA, USA). The immunoprecipitates were eluted and reverse crosslinked. DNA fragments were purified and quantitated using PCR amplification. For the analysis of the 1-cys Prx promoter, intron and exon regions, the following PCR primers were designed: promoter region (position -911 to -672 from translation start site), 5'-GTT GACCTGCACACAGTAGGTCTC-3' (forward) and 5'-CCT ACAGTGGAGTGGAGTGACTGCT-3' (reverse); final intron region (position 9,353 to 9,708), 5'-GTCATGGCTGTAAA GTACTGGTG-3' (forward) and 5'-CACTGGAATGGAAGT TCTATGAGGG-3' (reverse); final exon (position 9,989 to 10,347), 5'-GCTTGGAGAAGAAGCTGCAGAA-3' (forward) and 5'-CTATCCCCATCCTATTGAAAGAC-3' (reverse).

Results and Discussion

Transcriptional regulation of 1-cys Prx by DEK. Previously, we identified differential proteomic profiles in DEK knockdown HeLa cells (9), among them the regulated protein 1-cys Prx, which was up-regulated. The regulatory mechanism of 1-cys Prx was further investigated in this study. To investigate whether DEK regulates the transcription of 1-cys Prx, we performed a luciferase reporter assay, in which the human 1-cys Prx promoter region (positions -51 to -1,501) was cloned

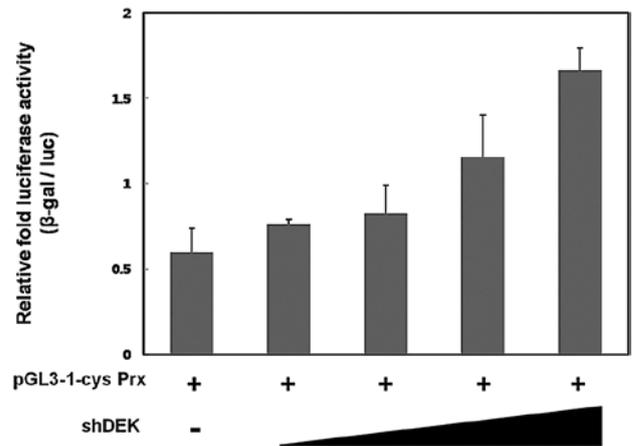


Figure 1. Knockdown of endogenous DEK enhances 1-cys Prx transcriptional activity. HeLa cells were transfected with 1-cys Prx reporter (100 ng) and shDEK (0, 50, 100, 150 or 200 ng). Relative luciferase activity was determined at 48 h post-transfection. Bars represent the average of three independent assays and error bars represent the \pm SD.

into pGL3-basic vector. The transcriptional activity of 1-cys Prx was initially measured, and an increasing amount of shDEK was co-transfected. As expected, the knockdown of DEK enhanced 1-cys Prx transactivation in a dose-dependent manner (Fig. 1). This reporter assay indicates that the transcription of 1-cys Prx is regulated by DEK.

C-terminus HAT inhibitory domains are responsible for DEK-mediated transcriptional repression of 1-cys Prx. We previously demonstrated that DEK inhibits histone acetylation by p300/CBP or PCAF and directly binds to histones (2). Moreover, the acidic domains of DEK are critical for HAT inhibitory activity and histone binding affinity. To further investigate the functional role of acidic domains in the transcriptional regulation of 1-cys Prx, reporter assays were performed using DEK deletion mutants. A series of C-terminal acidic domain deletion mutants, DEK-ΔC290 and DEK-ΔC200, were used in the reporter assay (Fig. 2A). Recently, we showed that the deletion mutant DEK-ΔC200, which has lost the C-terminus acidic domains, showed very weak HAT inhibitory activity (Fig. 2A) (2). Consistent with the finding that shDEK mediated transactivation, the overexpression of wild-type DEK significantly repressed 1-cys Prx transcriptional activity (Fig. 2B). Transient transfection of DEK-ΔC290 reversed DEK-mediated transcriptional repression, and DEK-ΔC200, which lost both C-terminal acidic domains, further rescued the repression of 1-cys Prx (Fig. 2B). These results are consistent with the acidic domains-mediated HAT inhibitory and transcriptional repression activities of DEK, and indicate the importance of acidic domains in the C-terminus.

Transcriptional repression of 1-cys Prx by the p65 subunit of NF-κB. Bioinformatical analysis was performed with the 1-cys Prx promoter region using TESS (<http://www.cbil.upenn.edu/cgi-bin/tess/tess>) to identify the transcription factors that affect 1-cys Prx gene expression. By a computational search, the predicted NF-κB binding site in the

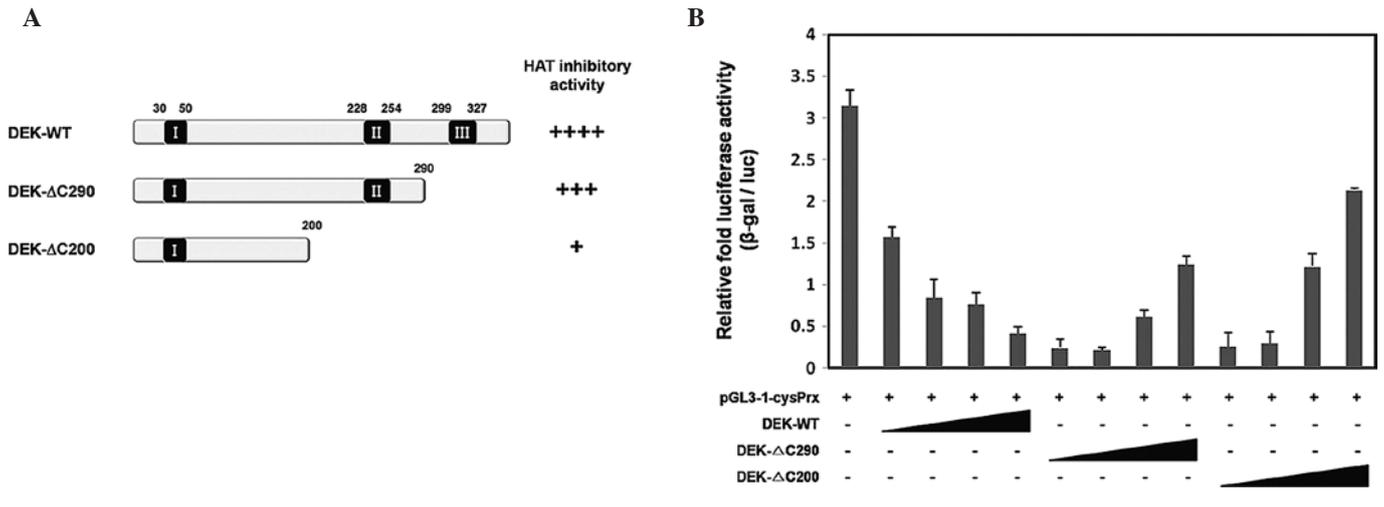


Figure 2. The C-terminus acidic domain of DEK is key in the transcriptional repression of 1-cys Prx. (A) Schematic diagram of DEK deletion mutants. Black boxes indicate acidic domains in the DEK protein. The HAT inhibitory activity of each mutant is indicated. Briefly, HAT inhibitory activity is also classified on a scale from ++++ (high activity) to + (little activity, ~20% of wild-type). (B) HeLa cells were transfected with DEK wild-type (DEK-WT) and the deletion constructs DEK-ΔC290 and -ΔC200 (50, 100, 200 and 300 ng each) together with 1-cys Prx reporter (100 ng). Relative luciferase activity was determined at 48 h post-transfection. Bars represent the average of three independent assays and error bars represent the ± SD.

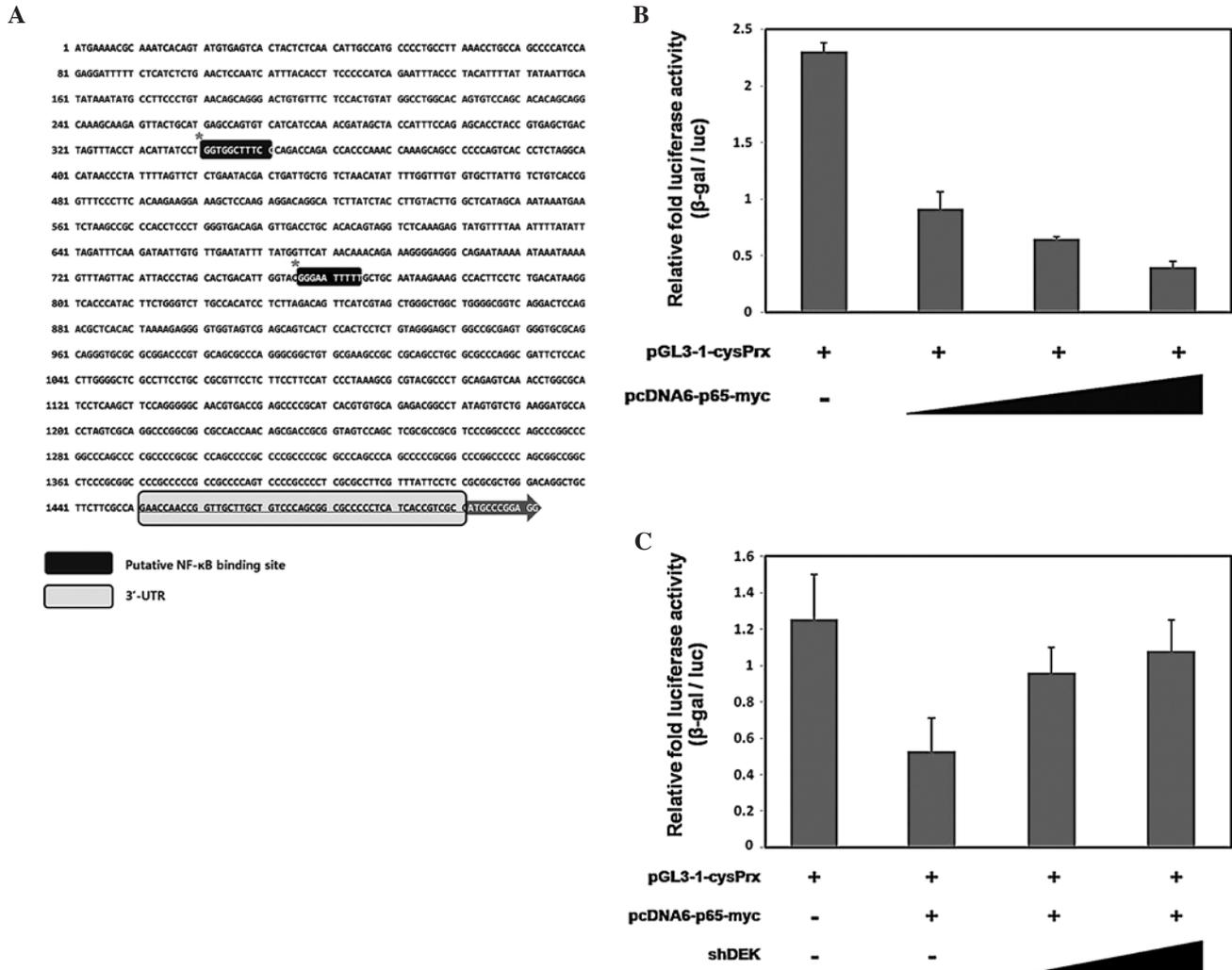


Figure 3. Transcriptional repression of 1-cys Prx by p65 and DEK. (A) The asterisks indicate the putative binding sites, and the arrow box designates the coding region of 1-cys Prx as determined by the TESS program. The underlined sequence in the box is the 3' untranslated region. (B) HeLa cells were transfected with 1-cys Prx reporter (100 ng) and pcDNA6-p65-myc (0, 50, 100 and 150 ng). Relative luciferase activity was determined at 48 h post-transfection. Bars represent the average of three independent assays and error bars represent the ± SD. (C) HeLa cells were transfected with pcDNA6-p65-myc (100 ng) and shDEK (50 and 100 ng) together with 1-cys Prx reporter (100 ng). Relative luciferase activity was determined at 48 h post-transfection. Bars represent the average of three independent assays and error bars represent the ± SD.

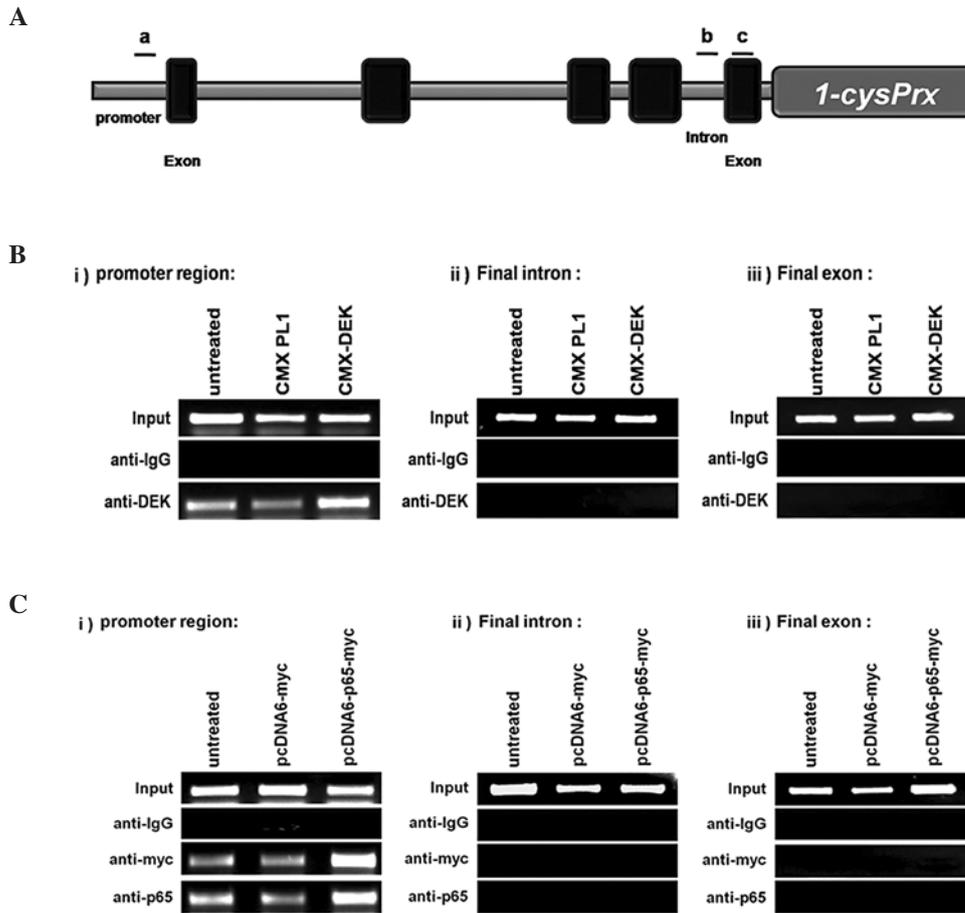


Figure 4. Occupancy of DEK and the p65 subunit in the 1-cys Prx promoter region. (A) Schematic diagram of the primer pairs used for ChIP analysis. Black boxes represent the 1-cys Prx exons and the gray bar indicates the intron. (B and C) HeLa cell lysates were transfected with CMX-PL1, CMX-DEK, pcDNA6-myc or pcDNA6-p65-myc and analyzed by ChIP using mouse IgG, anti-DEK antibodies (B) or mouse IgG, anti-p65 and anti-myc antibodies (C). The immunoprecipitated DNA was amplified by PCR using promoter (i), final intron (ii) and final exon (iii) specific primer pairs.

1-cys Prx promoter region was positioned at -737 to -746 from the transcriptional start site (Fig. 3A). This putative NF- κ B binding site is also present in the pGL3-1-cys Prx reporter vector. A previous report demonstrated that HDACs modulate the transcriptional activity of NF- κ B through protein-protein interaction (14). DEK was characterized as a p65 interacting protein by a yeast-two hybrid experiment, and shown to result in the inhibition of p65-mediated transcriptional activity (13). The fact that DEK interacts with p65 and that both proteins are involved in transcriptional repression prompted us to investigate whether p65 protein has a role in DEK-mediated 1-cys Prx transcriptional repression. The reporter assay was first carried out with 1-cys Prx promoter and pcDNA6-p65-myc. Increasing concentrations of p65 caused the transcriptional activity of 1-cys Prx to be down-regulated (Fig. 3B).

NF- κ B regulates many cellular processes through the transcriptional activation of target genes. However, Shaw *et al* suggested that NF- κ B may also act as a transcriptional repressor via interaction with HDACs (15). Transcriptional repression of 1-cys Prx by p65 was reversed by co-transfection with shDEK (Fig. 3C). Our results indicate that p65 and DEK have a synergistic effect on the transcriptional repression of 1-cys Prx.

Recruiting of DEK and p65 in the 1-cys Prx promoter. To further demonstrate that both DEK and p65 are recruited to the 1-cys Prx promoter region, a ChIP assay was performed. As expected, when DEK and p65 were overexpressed, the 1-cys Prx promoter region was occupied by both DEK and p65 as compared to untreated and empty vector transfected cells (Fig. 4B-i and C-i). By contrast, the 1-cys Prx final intron and final exon were not occupied by either protein (Fig. 4B-ii and iii and C-ii and iii). These ChIP assay results indicate that both the DEK and p65 proteins are recruited to the 1-cys Prx promoter region and negatively regulate its transcription. It is possible that p65 binds directly to DNA and recruits DEK protein as a transcriptional repressor through protein-protein interaction.

In this study, we identified the functional role of DEK in 1-cys Prx transcription. DEK functions as a transcriptional repressor of 1-cys Prx by cooperating with the p65 subunit of NF- κ B. Further studies are required to elucidate the detailed mechanisms of DEK and p65-mediated transcriptional regulation of 1-cys Prx, and its physiological effects.

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