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Chromatin Redistribution of the DEK Oncoprotein Represses *hTERT* Transcription in Leukemias[1,2]

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Abstract

Although numerous factors have been found to modulate *hTERT* transcription, the mechanism of its repression in certain leukemias remains unknown. We show here that DEK represses *hTERT* transcription through its enrichment on the *hTERT* promoter in cells from chronic and acute myeloid leukemias, chronic lymphocytic leukemia, but not acute lymphocytic leukemias where *hTERT* is overexpressed. We isolated DEK from the *hTERT* promoter incubated with nuclear extracts derived from fresh acute myelogenous leukemia (AML) cells and from cells expressing Tax, an *hTERT* repressor encoded by the human T cell leukemia virus type 1. In addition to the recruitment of DEK, the displacement of two potent known *hTERT* transactivators from the *hTERT* promoter characterized both AML cells and Tax-expressing cells. Reporter and chromatin immunoprecipitation assays permitted to map the region that supports the repressive effect of DEK on *hTERT* transcription, which was proportionate to the level of DEK-promoter association but not with the level of DEK expression. Besides *hTERT* repression, this context of chromatin redistribution of DEK was found to govern about 40% of overall transcriptional modifications, including those of cancer-prone genes. In conclusion, DEK emerges as an *hTERT* repressor shared by various leukemia subtypes and seems involved in the deregulation of numerous genes associated with leukemogenesis.

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Introduction

The majority of human tumor cells possess shorter telomeres than their normal counterparts, suggesting that abnormal telomere shortening is frequently involved in cancer [1–3]. The cellular reverse transcriptase telomerase counteracts telomere shortening. This enzyme is composed of a catalytic protein subunit, telomerase reverse transcriptase (*hTERT*), and an RNA template (hTR). Malignant cells regularly bypass replicative senescence and paradoxically combine high telomerase activity with short telomere length [1]. However, the positive correlation between *hTERT* overexpression, increased telomerase activity, and oncogenesis does not seem to be mandatory in all tumor cases because *hTERT* underexpression has been shown at some stages of chronic myeloid leukemia (CML) [4,5], adult T cell leukemia/lymphoma [6–8], chronic lymphocytic leukemia (CLL) [9], and acute myelogenous leukemia (AML) [10]. To date, *hTERT* transcriptional repression has been considered as a tumor-suppressor pathway [11], and in contrast to transcriptional *hTERT* activation, very little is known about how *hTERT* is transcriptionally repressed in some hematological malignancies.

The protein DEK was originally identified as a fusion with the CAN/NUP214 nucleoporin in a subset of AML patients who harbored the (6;9)(p23;q64) translocation and was subsequently found overexpressed in most AMLs, as in numerous solid tumors [12]. DEK-CAN induces leukemia in mouse models [13], while its role of DEK in transcription varies on the basis of cell type, gene target, and developmental context. DEK enhances the transcription capacity of AP-2 in human malignant glioblastoma [14] and acts as a co-activator of the nuclear splicing factor U2AF in HeLa cells [15]. However, DEK acts as a co-repressor on p65/nuclear factor κB [16]. More recently, Koleva et al. showed that through their chromatin redistribution, DEK and C/EBPα cooperate together to coordinately activate myeloid gene expression and thereby regulate the differentiation capacity of hematopoietic progenitors [17].

We conducted the present study to assess how *hTERT* is transcriptionally repressed in certain leukemias. We designed a magnetic promoter precipitation assay coupled with mass spectrometry (MPP-MS) to identify proteins bound to the *hTERT* promoter in various cell types. We and others previously found that the oncoprotein Tax encoded by the human T cell leukemia virus type 1 (HTLV-1) represses *hTERT* transcription [6,7,18,19] in proliferating cells, whereas it activates *hTERT* expression in quiescent cells [7,20]. We therefore hypothesized that leukemic cells with low hTERT expression and Tax-expressing cells might share similar mechanisms of hTERT repression. Using a Tax-based system of *hTERT* transcriptional repression [6], we first demonstrated that Tax displaces transactivators from the *hTERT* promoter, where it recruits DEK that we subsequently characterized as an *hTERT* transcriptional repressor. Furthermore, the data indicate that Tax-expressing cells and fresh AML cells shared numerous common changes of the *hTERT* promoter proteome including DEK recruitment. Given that DEK is a chromatin protein deregulated in leukemias, we then begun to investigate whether or not DEK was involved in the deregulation of additional Tax-targeted genes as in the repression of *hTERT* in HTLV-1–unrelated leukemias.

Materials and Methods

The detailed materials and methods are described in the Supplementary Materials and Methods.

Cell Material

After consent was obtained in accordance with the Declaration of Helsinki and institutional guidelines, bone marrow (BM) cells were obtained from 6 donors and 20 patients (Table W1). $CD34^+$ cells were isolated from mononuclear cells using immunomagnetic microbeads and the Dynal CD34 progenitor cell selection system (Dynal Biotechnologies, Oslo, Norway). B-lymphocytes were purified by negative selection using the RosetteSep human B cell enrichment cocktail (STEMCELL Technologies, Grenoble, France). HeLa cells were obtained from the European Collection of Cell Cultures (ECCAC, Salisbury, United Kingdom).

Plasmids, Transient Transfection, Immunoprecipitation, and Western Blot Analysis

HeLa and Jurkat cells were transiently transfected using the calcium phosphate precipitation method (CalPhos Transfection Kit; Clontech, Shiga, Japan) and SuperFect reagent (Qiagen, Courtaboeuf, France), respectively. Lipofectamine RNAi Max (Invitrogen, Carlsbad, CA) was used as the small interfering RNA (siRNA) delivery system. The plasmid vectors pTERTluc800, pCMV-Tax, pCMV-USF2a, and pNGLV3-DEK were previously described [6,21–24]. The corresponding empty vectors were used as controls. DEK siRNA was purchased from Dharmacon (Lafayette, CO). The co-immunoprecipitation experiments were carried out using NP-40 incubation buffer (150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1% NP-40, 50 mM *N*-ethylmaleimide, 2 mM EDTA, and protease inhibitors, 1:200, P8340 Sigma, St Louis, MO) and Protein G Sepharose Fast Flow (Sigma). Antibodies are detailed in the Supplementary Materials and Methods.

Quantitative Chromatin Immunoprecipitation and Quantitative Reverse Transcription–Polymerase Chain Reaction

The isolation, sonication, and chromatin immunoprecipitation (ChIP) analysis of nucleoprotein complexes of HeLa cells and leukemic cells are detailed in the Supplementary Materials and Methods. Quantitative reverse transcription–polymerase chain reaction (qRT-PCR) assays were performed on a LightCycler 2.0 system (Roche Applied Science, Indianapolis, IN) using the SYBR Green qPCR SuperMix UDG kit as detailed in the Supplementary Materials and Methods. The expression of each gene of interest was normalized against two housekeeping genes, *Gus* (NM_000181) and *HPRT* (NM_000194). All controls or samples were analyzed in duplicate. Primer sequences are provided in the Supplementary Materials and Methods.

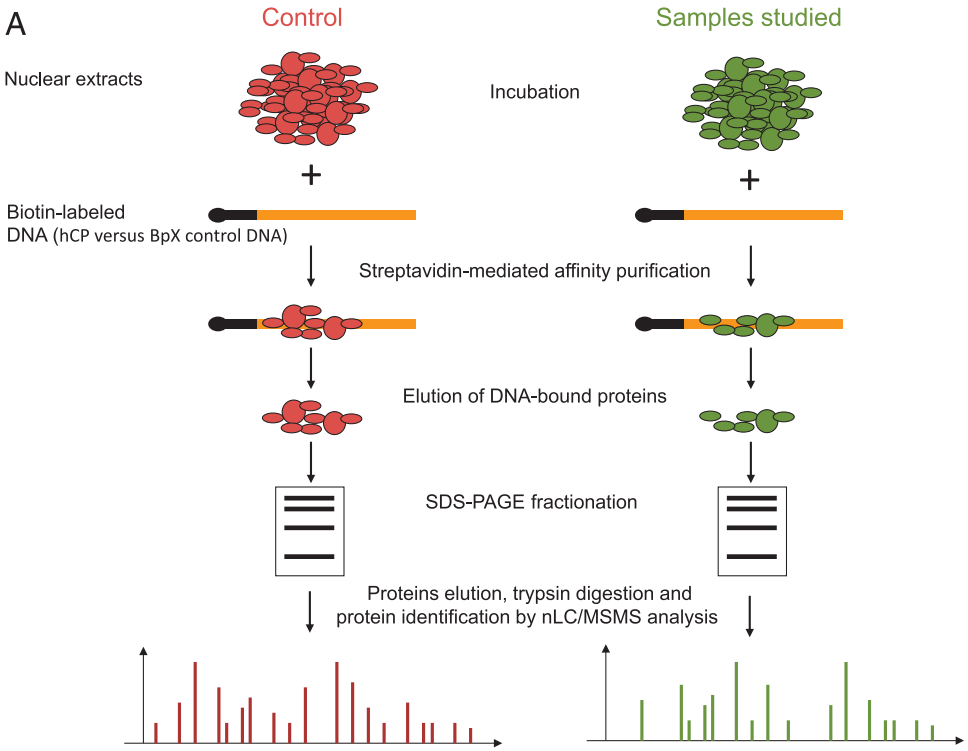
Array Hybridization and Processing

After quality control, total RNA was amplified and biotin-labeled by a round of *in vitro* transcription. It was then fragmented and hybridized. Slides were scanned and the image files were analyzed using CodeLink expression software. The microarray analyses consisted of statistical comparison and filtering using GeneSpring software 7.3.1 (Agilent Technologies, Santa Clara, CA). See Supplementary Materials and Methods.

Proteomic Analysis of hTERT *Promoter Occupancy* In Vivo

Biotinylated *hTERT* core promoter (hCP) was amplified by PCR from the pTERTLuc800 plasmid [6]. The control template corresponded to a PCR-generated fragment of the HTLV-1 provirus pX

region. Streptavidin beads were incubated with biotinylated templates. The beads/DNA complexes were washed, and immobilized templates were freshly prepared before each experiment. Finally, reaction components were incubated for 40 minutes at room temperature with 400 µg of dialyzed nuclear extracts (NEs). After washing, DNA template–specific proteins were eluted and resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). See Supplementary Materials and Methods.



Proteins shared by Tax-positive cells, Tax-negative cells, normal bone marrow CD34+ cells, and Patients' myeloblasts	
DDB1_HUMAN (Q16531)	DNA damage-binding protein 1 (Damage-specific DNA binding protein 1) (DDB p127 subunit) (DDBa) (UV-d
H2BA_HUMAN (P62807)	Histone H2B.a/g/h/k/l (H2B.1 A) (H2B/a) (H2B/g) (H23/h) (H23/k) (H2B/l)
EF1A1_HUMAN (P68104)	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Elongation factor 1 A-1) (eEF1A-1) (Elongation factor Tu
MYH9_HUMAN (P35579)	Myosin heavy chain, nonmuscle type A (Cellular myosin heavy chain, type A) (Nonmuscle myosin heavy c
ILF3_HUMAN (Q12906)	Interleukin enhancer-binding factor 3 (Nuclear factor of activated T-cells 90 kDa) (NF-AT-90) (Doubl
H4_HUMAN (P62805)	Histone H4
ILF2_HUMAN (Q12905)	Interleukin enhancer-binding factor 2 (Nuclear factor of activated T-cells 45 kDa)
VIME_HUMAN (P08670)	Vimentin
TPM4_HUMAN (P67936)	Tropomyosin alpha-4 chain (Tropomyosin-4) (TM30p1)
Proteins shared by Tax-negative cells and normal bone marrow CD34+ cells but not by patients' myeloblasts	
HNRPD_HUMAN (Q14103)	Heterogeneous nuclear ribonucleoprotein D0 (hnRNP D0) (AU-rich element RNA binding protein 1)
MSH2_HUMAN (P43246)	DNA mismatch repair protein Msh2
RIB1_HUMAN (P04843)	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit precursor (EC 2.4.1.11
Proteins shared by Tax-positive cell, patients' myeloblasts but not by Tax-negative cells or normal bone marrow CD34+ cells	
DEK_HUMAN (P35659)	Protein DEK
NHPX_HUMAN (P55769)	NHP2-like protein 1 (High mobility group-like nuclear protein 2 homolog 1) (U4/U6.U5) tri-snRNP 15.
RUXE_HUMAN (P62304)	Small nuclear ribonucleoprotein E (snRNP-E) (Sm protein E) (Sm-E) (SmE)
TOP1_HUMAN (P11387)	DNA topoisomerase I (EC 5.99.1.2)

Figure 1. Proteomic analysis of *hTERT* promoter occupancy. (A) Nuclear protein extracts were prepared either from HeLa cells transfected with a Tax-expressing plasmid (right) or from fresh BM AML tumor cells. HeLa cells transfected with the control empty plasmid and normal BM $CD34^+$ cells served as controls (left). Proteins were incubated with biotin-labeled DNA probes that corresponded to the *hCP* or to a control DNA stretch (*BpX*), as detailed in the Materials and Methods section. After SDS-PAGE fractionation, eluted products were digested with trypsin and then analyzed by MS. (B) The Venn diagram (top) represents the distribution of proteins detected in Tax^+ *versus* Tax^- NEs analyzed as shown in A. Data correspond to proteins recurrently detected in three independent experiments. Isolated proteins are described in Table W2. (Bottom) Distribution of *hTERT* promoter partners shared between transfected HeLa cells and BM cells derived from donors or from patients with AML.

Nano-Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC–MS/MS) Analysis

The method consisted of a 60-minute gradient at a flow rate of 200 nl/min, using a gradient of two solvents. MS and tandem mass spectrometry (MS/MS) data were acquired and processed automatically. Consecutive searches against, first, a contaminant database and then against the SwissProt and TREMBL databases were performed for each sample. See Supplementary Materials and Methods.

Statistics

Associations between categorical variables were analyzed by Fisher exact tests. The central tendency differences between groups were compared with the Mann-Whitney or Kruskal-Wallis tests. Nonparametric linear correlations between characteristics were analyzed by the Spearman rank test. All *P* values were two-sided.

Results

Tax-Expressing Cells and Fresh Myeloblasts Share Common hTERT *Promoter Proteome Modifications*

After validation of MPP assays as a reliable tool for isolation of specific *hTERT* promoter partners (Supplementary Results and Figure W1), our MPP-MS assays (Figure 1*A*) permitted to isolate 268 hCP-bound proteins from NEs deriving from Tax^- or Tax^+ cells (Figure 1*B* and Table W2). Of these, 22 and 18 were identified in NEs derived from Tax^- and Tax^+ cells, respectively. As shown in Figure 1*B* (*bottom*) and Table W2, myeloblast- and normal $CD34^+$-derived NEs shared common *hTERT* promoter partners with Tax^+- and/or Tax^--derived NEs. Remarkably DEK, TOP1, NHPX, and RUXE were eluted from hCP incubated with Tax^+- and myeloblast- but not from Tax^-- and normal $CD34^+$-derived NEs (Table W2). In contrast, MSH2, hnRNP D0, and RIB1 were eluted from hCP incubated with Tax^-- and $CD34^+$- but not from Tax^+- and myeloblast-derived NEs. These similarities suggested that, although occurring in different phenotypic contexts, both leukemic processes, i.e., AML and adult T cell leukemia, might share common mechanisms of *hTERT* repression.

hTERT *Promoter Repression Coincides with the Displacement of* hTERT *Transactivators and the Recruitment of DEK*

To validate MS data, we assessed the *hTERT* promoter occupancy *in vivo* for some MPP-eluted factors. Among those specifically isolated from Tax^- and normal $CD34^+$ cells, but not from Tax^+ and AML NEs, we selected the proteins MSH2 and hnRNP D0 because they have been previously reported to activate *hTERT* transcription through binding two distinct regions on the *hTERT* promoter in oral squamous cell carcinoma cells [25]. Quantitative ChIP (qChIP) assays showed that the relative amounts of immunoprecipitated *hTERT* promoter fragments were lower in Tax^+ than in Tax^- cells (2.2- and 1.4-fold for hnRNPD0 and MSH2, respectively; Figure 2). Among the hCP-bound proteins specifically shared by Tax^+- and AML-hCP proteomes (Table W2), we paid particular attention to the proto-oncogene DEK due to its important role in modifying the chromatin topology and gene expression during cell differentiation and transformation [26]. DEK occupancy of *hTERT* promoter was estimated by qChIP with anti-DEK antibody and seven primer sets spanning the hCP (Figure 3*A*). Figure 3*A* shows that DEK-DNA association was higher in Tax-expressing cells. In addition, the amount of amplified DNA varied along the immunoprecipitated *hTERT* promoter from Tax^+ cells where the intensity peaked at 9.46% input with the TERT6 primer set that overlaps the transcription start site (Figure 3*A*). The effect of DEK on *hTERT* transcription was then assessed through reporter assays. Figure 3*B* shows that pNGLV3-DEK inhibited TERTLuc800 expression in a concentration-dependent manner and that Tax expression showed an additive effect on this repression. Conversely, siRNA-mediated knockdown of DEK expression increased *hTERT* transcription and abolished the negative effect of Tax on endogenous *hTERT* expression in HeLa cells (Figure 3*C*). Change in DEK expression level was also found to modulate endogenous *hTERT* expression in Jurkat cell lines (not shown).

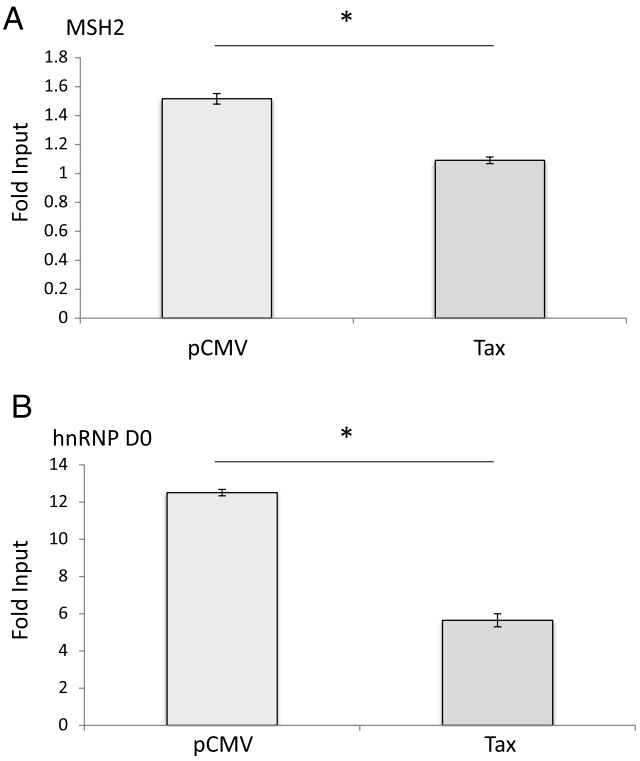


Figure 2. hnRNP D0 and MSH2 are displaced from the *hTERT* promoter upon Tax expression. Analysis of MSH2 (A) and hnRNP D0 (B) associations with the *hTERT* promoter was carried out by qChIP with HeLa cells expressing or not Tax, using antibodies against hnRNP D0 and MSH2 and qPCR with PCR primers complementary to the *hTERT* promoter, as described in the Materials and Methods section. qChIP assays were performed in triplicates with $P < .05$ (*) tested with the one-tailed Mann-Whitney test.

In Fresh Leukemic Cells, DEK Recruitment Parallels hTERT *Transcriptional Repression*

The above results prompted us to investigate whether the DEK-mediated transcriptional repression of *hTERT* pertains to other leukemic contexts. To this end, *hTERT* transcripts were quantified by qPCR in cells derived from CML, acute lymphoblastic leukemia (ALL), and CLL patients (Table W1) and in normal BM mononuclear cells (BMMNCs) and purified B cells used as controls. In parallel, the fold enrichment of DEK at the *hTERT* promoter DNA sequences was assessed by qChIP assays in the same samples (Figure 4). The mean amounts of *hTERT* transcripts for AML, CML, ALL, and CLL values were 0.28, 0.41, 0.98, and 0.26 arbitrary units, respectively, while those

of normal BMMNCs, purified BM CD34$^+$ cells, and purified peripheral B cells were 0.76, 0.73, and 0.72 arbitrary units, respectively. This confirmed previous results showing that *hTERT* expression is increased in ALL and decreased in CML, CLL, and AML [4–7,9,10,19]. The mean fold enrichment of DEK at the *hTERT* promoters in normal BMMNCs, normal BM purified CD34$^+$ cells, AML, CML, ALL, normal purified circulating B cells, and CLL samples were 0.44, 0.56, 1.41, 1.74, 0.72, 0.68, and 1.76, respectively ($P = .014$, Kruskal-Wallis test). Samples with the highest DEK-*hTERT* association displayed the lowest amounts of *hTERT* transcripts, and by linear regression analysis, a significant negative correlation linked these two values (Figure 4*A*; $P = .00002$, $R \sim -0.75$, Spearman rank correlation). In contrast, Figure 4*B* shows that *in vivo*, DEK mRNA levels were widely dispersed over cell samples without any statistical correlation between *DEK* and *hTERT* mRNA levels ($P = .26$, $R \sim -0.21$, Spearman rank correlation). Similarly, DEK expression remained unchanged upon Tax expression (Figure 4*C*).

hTERT *Repression Is Influenced by DEK Posttranslational Modification*

Both phosphorylation and acetylation decrease DEK's DNA affinity [16] and thereby modulate its transcriptional effects [27]. To address the function of DEK phosphorylation on *hTERT* expression, we generated a DEK phosphorylation mutant, 4ADEK, by introducing alanine substitutions at serines 301, 303, 306, and 307. These residues were chosen because they are involved in DEK-DNA binding [28,29]. After transfection, the overall DEK amounts were unchanged between assays using either 4ADEK- or the wild-type (WT)–DEK-expressing vectors (not shown). In contrast, the suppressive effect of the 4ADEK mutant on the *hTERT* transcription was three times higher than that of WT-DEK (Figure 5*A*). In parallel, qChIP revealed that the 4ADEK-*hTERT* DNA association was higher than that obtained with the WT-DEK plasmid (Figure 5*B*). These results strongly supported that DEK-DNA association governed DEK-dependent *hTERT* transcriptional repression and suggested that DEK phosphorylation was critical for these processes. To investigate the relationship between DEK acetylation and *hTERT* expression, we assessed the amount of acetylated DEK in Tax$^-$ and Tax$^+$ NEs. Figure 5*C* shows that Tax

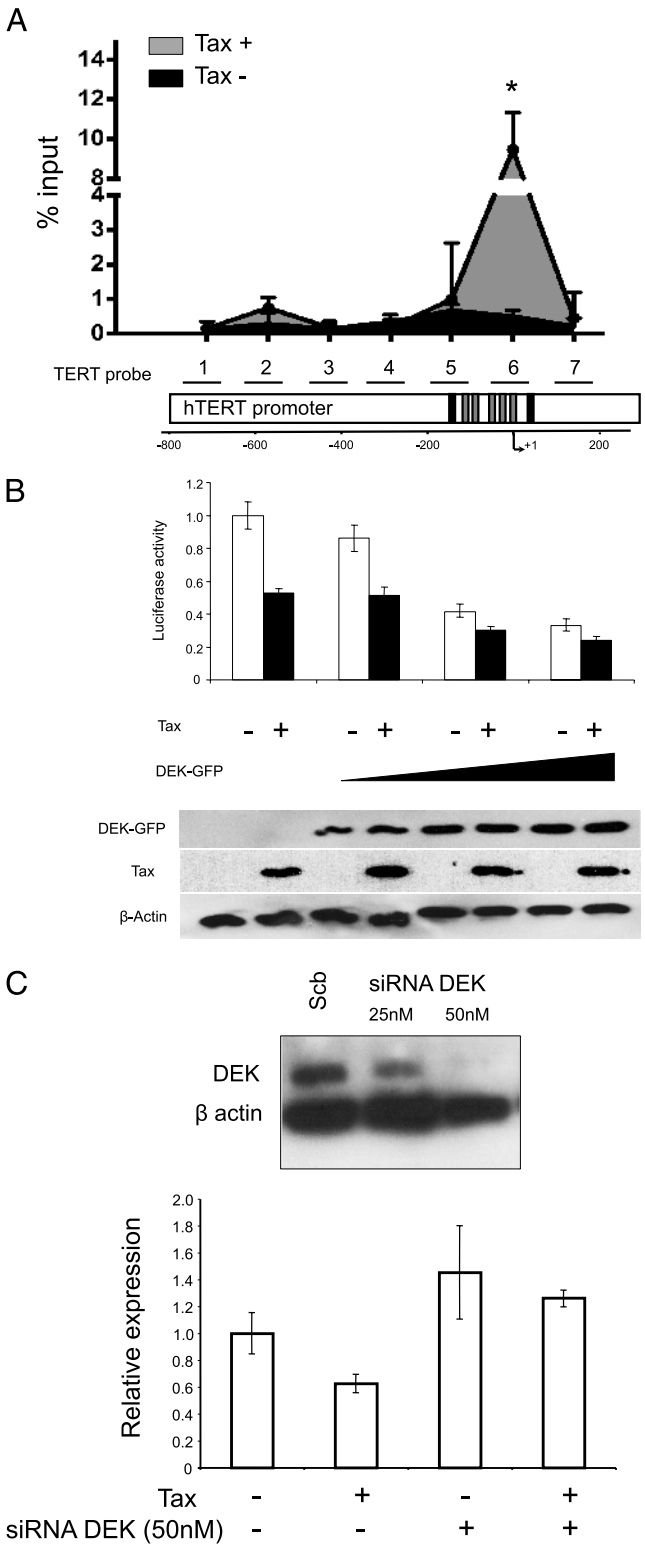


Figure 3. DEK binds to and suppresses the *hTERT* promoter on Tax expression. (A) DEK and *hTERT* promoter association in Tax-expressing cells. The seven primer pairs used for ChIP and qChIP spanned a 964-bp region of the *hTERT* promoter. This region encompasses several known critical sites involved in the regulation of *hTERT* transcription, including the transcription initiation site, the start codon, as well as GC and E boxes. The black boxes represent the E boxes, while the gray boxes represent the five Sp1 binding sites. +1 is the transcription initiation site. qChIP analysis of DEK association with the *hTERT* promoter was carried out as described in the Materials and Methods section. Results (means ± SDs) are representative of triplicate experiments. *$P < .05$, Mann-Whitney test. (B) HeLa cells were co-transfected with WT *hTERT* promoter–luciferase reporter plasmid TERTLuc800, in combination with the pCMV-Tax plasmid and/or a control vector (pCMV) in the absence or presence of increasing amounts of pNGLV3-DEK. Forty-eight hours after transfection, HeLa cells were collected and transcriptional activity was assayed by luciferase activity (see Materials and Methods section). (Bottom) DEK and Tax expression in transfected HeLa cells were assayed by Western blot analysis. (C) DEK knockdown increased endogenous *hTERT* expression and prevented its repression by Tax. *hTERT* expression was quantified through quantitative real-time PCR in HeLa cells transfected with the pCMV-Tax plasmid and/or control vector and/or the DEK siRNA (50 nM) and/or scrambled RNA. siRNA-mediated knockdown of DEK expression was checked by Western blot analysis (top). Data shown in B and C are the means (±SDs) of one representative experiment performed in triplicate.

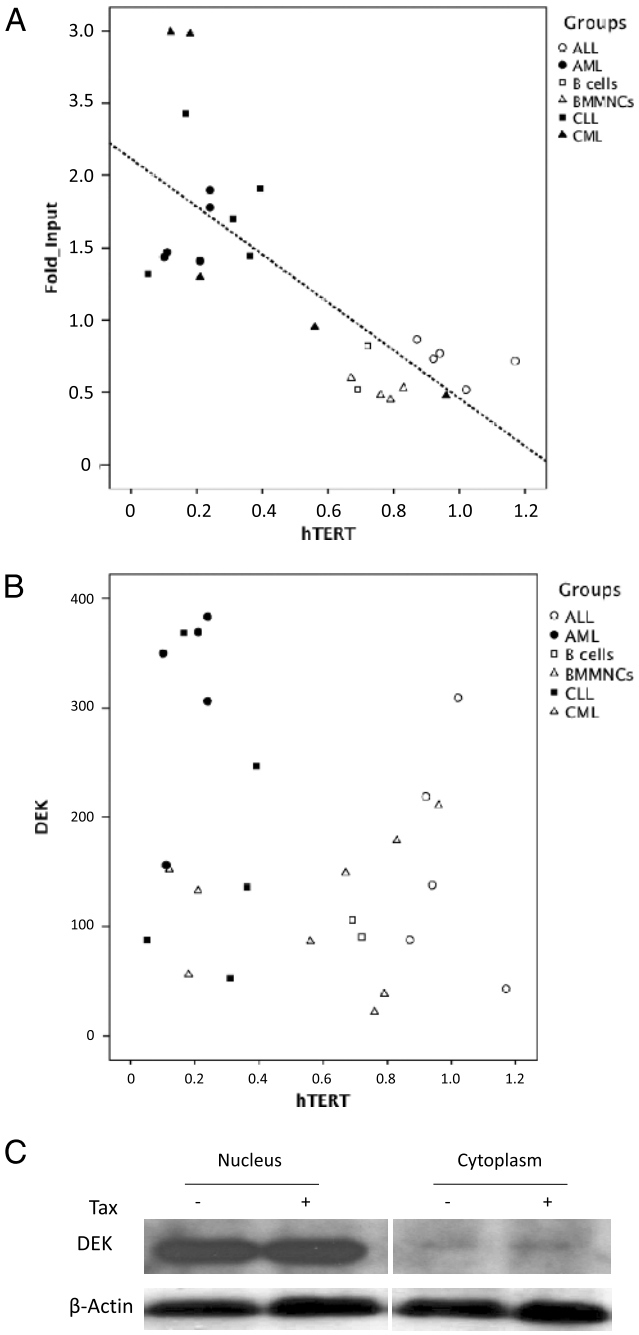


Figure 4. *In vivo* and *ex vivo*, DEK recruitment on the endogenous *hTERT* promoter correlates with hTERT transcriptional repression but not with DEK expression. (A) Analysis of DEK association with the *hTERT* promoter was carried out by qChIP using an antibody against DEK and qPCR with the TERT6 primer pair as described in the Materials and Methods section. For each sample, *hTERT* expression was measured by qRT-PCR. AML, CML, and ALL cells and BMMNCs were derived from the BM, while purified B cells and malignant CLL cells were derived from the blood. All patient samples were collected at the time of diagnosis, after written informed consent. Signals were normalized to input, and background levels in immunoprecipitation (IP) with control IgGs were assigned. (B) DEK expression did not correlate with *hTERT* transcriptional repression in hematological samples. For each sample, DEK and *hTERT* mRNA were quantified by qRT-PCR. (C) Tax expression did not modify DEK expression. NEs and cytoplasmic extracts from Tax- *versus* control empty vector–transfected HeLa cells were analyzed by Western blot analysis with an anti-DEK antibody.

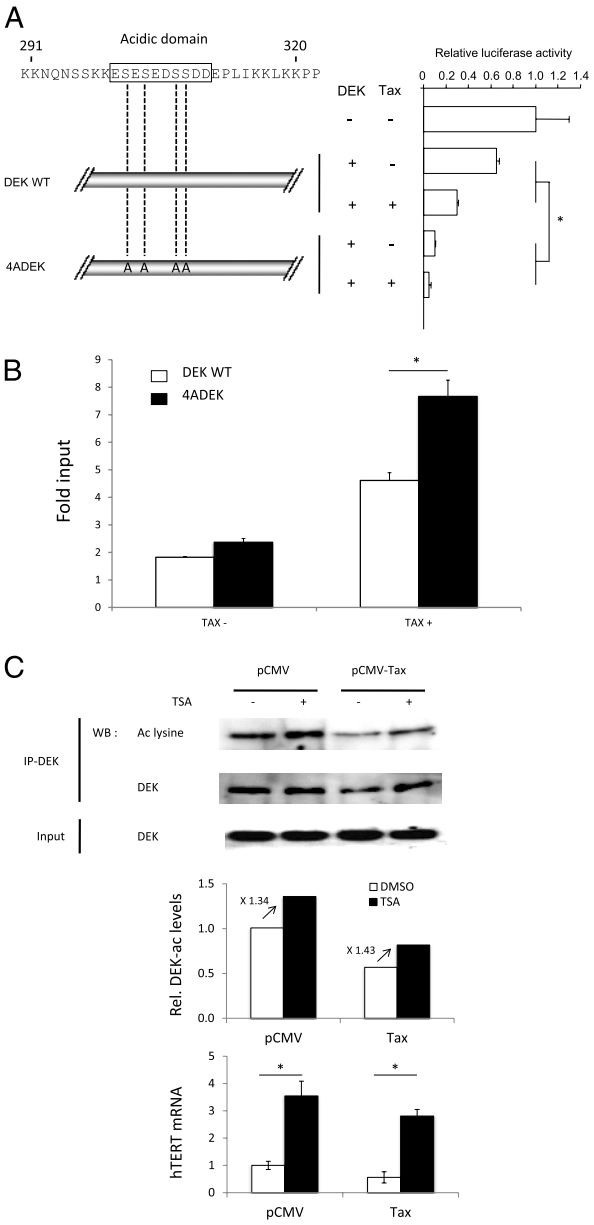


Figure 5. Transcriptional repression of *hTERT* depend on posttranslational modifications of DEK. (A) Mutations of the DEK phosphorylation site increase the repressive effect of DEK and Tax on *hTERT* promoter activity. HeLa cells were co-transfected with the WT *hTERT* promoter–luciferase reporter plasmid TERTLuc800, in combination with either the pCMV-Tax plasmid, the pNGLV3-DEK WT, or the pNGLV3-4ADEK mutant. Transcriptional activity was assayed by luciferase activity (see Materials and Methods section). (B) Mutations of the DEK phosphorylation site increased the recruitment of DEK on the *hTERT* promoter. qChIP was carried out with an anti-DEK antibody and the TERT6 primer pair, and nuclear proteins were derived from HeLa cells co-transfected with either the pCMV-Tax plasmid, the pCMV control vector, the pNGLV3-DEK WT, or the pNGLV3-4ADEK mutant. (C) Tax decreased DEK acetylation in a TSA-independent manner. HeLa cells were transfected with Tax or the control empty pCMV plasmid in the presence of TSA or DMSO and subjected to immunoprecipitation and Western blot analysis for DEK. The Western blot analysis membrane was stripped and reprobed with an anti-acetylated lysine antibody. The endogenous expression of *hTERT* was measured by qRT-PCR. Error bars represent the S.D. in triplicate experiments. Data shown in B and C are the means (±SDs) of one representative experiment performed in triplicate. *$P < .05$, Mann-Whitney test.

Table 1. Distribution of Gene Expression according to Tax Expression and DEK Knockdown.

Effect of DEK Knockdown	Tax-Activated (%)	Tax-Repressed (%)	Tax-Unmodified (%)
Activation	539 (13)	2029 (44)	1991 (9)
Repression	1613 (40)	644 (14)	3235 (14)
None	1898 (47)	1895 (42)	18101 (77)

decreased DEK acetylation without modifying its expression. In contrast, the exposure of HeLa cells to the deacetylase inhibitor trichostatin A (TSA) led to a decrease in DEK occupancy at the *hTERT* promoter (Figure W2), while it increased the amounts of both acetylated DEK and *hTERT* mRNA in HeLa cells expressing or not Tax

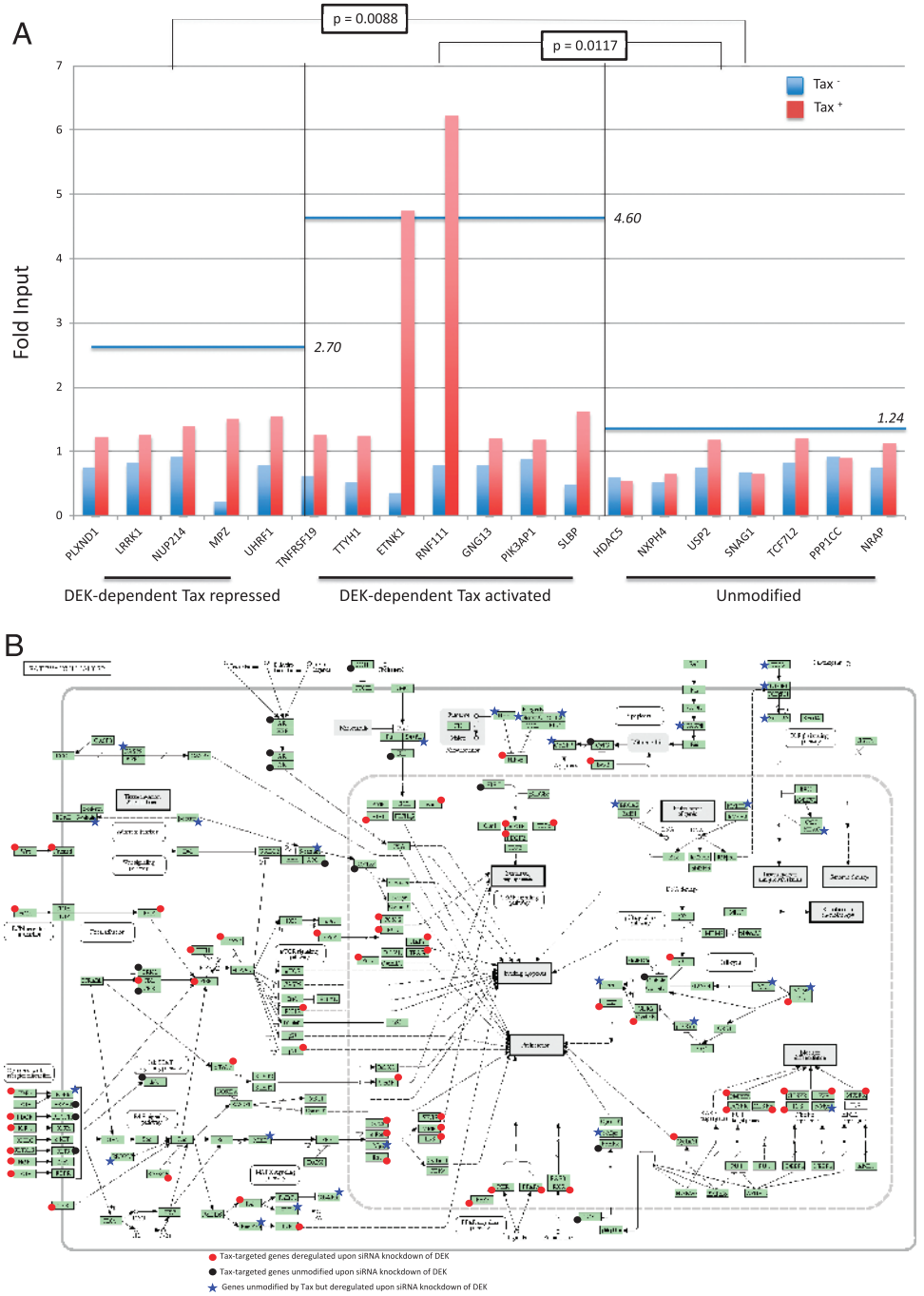


Figure 6. qChIP analysis of DEK-promoter association upon Tax expression for genes other than *hTERT*. (A) HeLa cells were transfected with either pCMV-Tax or control pCMV empty vector, and qChIP was carried out with a DEK antibody and PCR primers complementary to a promoter region encompassing the transcription start site of 19 genes. For each gene, the signal was normalized to input for Tax^+ (blue) and Tax^- HeLa cells (red). As indicated at the bottom, these genes included five DEK-dependent/Tax-repressed (left), seven DEK-dependent/Tax-activated (center), and seven DEK-independent/Tax-unmodified genes (right). For each gene category, a horizontal blue line represents the mean DEK-promoter association ratio of Tax^+ to Tax^- signals. (B) Distribution of "cancer" pathways according to the DEK-dependent (red circles) *versus* DEK-independent (black circles) nature of Tax-targeted genes. A blue asterisk identifies Tax-independent genes that were deregulated on siRNA knockdown of DEK.

Table 2. Ontological Analysis of DEK-Dependent and DEK-Independent Tax-Targeted Genes.

Terms	Gene Count	*P*
Enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of genes dysregulated by Tax expression in a DEK-dependent or DEK-independent manner		
hsa04080: neuroactive ligand-receptor interaction	63	9.193E–06
hsa04010: MAPK signaling pathway	59	4.504E–04
hsa05200: pathways in cancer	59	4.257E–02
hsa04060: cytokine-cytokine receptor interaction	52	1.052E–02
hsa04020: calcium signaling pathway	48	7.685E–06
hsa04514: cell adhesion molecules (CAMs)	26	7.960E–02
hsa04270: vascular smooth muscle contraction	25	2.403E–02
hsa05416: viral myocarditis	19	9.047E–03
hsa05414: dilated cardiomyopathy	19	9.702E–02
hsa05412: arrhythmogenic right ventricular cardiomyopathy (ARVC)	18	3.614E–02
hsa05410: hypertrophic cardiomyopathy (HCM)	18	8.922E–02
hsa04730: long-term depression	17	3.043E–02
hsa05218: melanoma	17	3.881E–02
hsa04260: cardiac muscle contraction	17	8.103E–02
hsa02010: ABC transporters	11	8.656E–02
Enriched KEGG pathways of genes dysregulated by Tax expression in a DEK-independent manner		
hsa04080: neuroactive ligand-receptor interaction	34	1.833E–04
hsa04010: MAPK signaling pathway	26	5.523E–02
hsa04020: calcium signaling pathway	23	3.228E–03
hsa04514: cell adhesion molecules (CAMs)	15	5.862E–02
hsa04340: hedgehog signaling pathway	9	3.217E–02

(Figure 5*C*). This suggests that DEK acetylation could counteract the transcriptional repression of *hTERT*. Typically, modifications in DEK acetylation could result from either Tax-induced deacetylase activity or Tax-induced inhibition of histone acetyltransferase activity. However, Figure 5*C* shows that TSA exposure did not alleviate the negative effect of Tax on DEK acetylation (Figure 5*C*), thereby ruling out the Tax-dependent deacetylation possibility. Therefore, it was possible to propose that the decreased amount of acetylated DEK mainly relies on a Tax-triggered histone acetyltransferase inhibition rather than on a Tax-triggered deacetylase effect. Taken together, these results demonstrated that DEK-dependent *hTERT* repression does not rely on increased DEK expression but rather on a greater DEK-DNA association, the level of which seems to be modulated at the post-translational level through DEK phosphorylation and acetylation.

Tax-Associated Cellular Gene Transcriptional Deregulation Frequently Depends on DEK

Given that DEK is an abundant chromatin protein in human cells, we investigated whether Tax-mediated chromatin redistribution of DEK might influence the transcription of other genes than *hTERT*. As detailed in the Supplementary Results and shown in Tables 1, W3, and W4, DEK was found involved in the transcriptional deregulation of 42% of Tax-targeted genes including 44% of Tax-repressed genes and 40% of Tax-activated genes. qChIP assays showed that Tax expression strengthened DEK recruitment on the promoter of 12/12 genes found to be transcriptionally modified by Tax in a DEK-dependent manner, including 5/5 Tax-repressed and 7/7 Tax-activated genes (Figure 6*A*). Furthermore, qChIP revealed that the average level of DEK-*hTERT* promoter association was higher for Tax-activated ($P = .0117$) and Tax-repressed ($P = .0088$) genes than for unmodified genes. By using the DAVID bioinformatics resources (http://david.abcc.ncifcrf.gov/), ontological analysis of the Tax-targeted genes showed that the two top-ranked term categories "Pathways in cancer" and "Cytokine-cytokine receptor interaction" were DEK-dependent (Supplementary Results, Tables 1, 2, and W4). In contrast, the terms "Neuroactive ligand-receptor interaction" and "MAPK signaling pathway" appeared independent of DEK. Figure 6*B* represents the "Pathways in cancer" and shows that the vast majority of Tax-targeted genes in these pathways are DEK-dependent genes.

Discussion

The study identified and characterized *hTERT* promoter partners involved in *hTERT* transcriptional repression in leukemias. Some of these partners such as MSH2 and hnRNP D0 were found displaced from the *hTERT* promoter in both AML cells and Tax-expressing cells, while other factors including DEK were found recruited to this promoter in both cell subtypes. Given its known implication in leukemias, we further detailed the role of DEK in *hTERT* transcription. DEK was found recruited on *hTERT* promoter not only in AML and Tax-expressing cells but also in CML and CLL cells. We found that DEK repressed *hTERT* transcription and that this repression relied on the recruitment of DEK on the *hTERT* promoter but not on the amounts of DEK protein in tumoral cells. Posttranslational DEK modifications were found to influence its interplays with *hTERT*. In addition to *hTERT*, DEK/promoter enrichment was also found to regulate more than 40% of Tax-controlled genes, including those having critical roles in cancer.

DEK has emerged as a novel class of DNA topology modulators that can be both targets and effectors of tumor initiation and addiction [12,16]. The proto-oncogene DEK is involved in chromatin remodeling, transcriptional repression or activation, and mRNA maturation [12,16]. DEK is capable to associate with numerous promoters for modulating their occupancy in transcriptional factors and chromatin modifiers such as hDaxx [30], P/CAF, p300, and p65/nuclear factor κB [26,31]. DEK prefers structured DNA, such as supercoiled and four-way junctions, to specific nucleic acid sequence [28]. Such structured DNA forms are enriched in the vicinity of promoter regions [32]. DEK and other cruciform-binding proteins such as p53, BRCA1, MSH2, PARP1, 14-3-3, and topoisomerase 1 are frequently involved in transcriptional regulation as well as in DNA repair and replication [33]. Interestingly, our results show that in addition to enrichment in the cruciform-binding protein DEK at the vicinity of the *hTERT* transcription start site, *hTERT* repression also included changes in occupancy of additional cruciform-binding proteins such as MSH2, hnRNPs, and topoisomerase 1 (Figure 1).

AML, CML, and CLL cells display significantly lower amounts of *hTERT* transcripts than their normal counterparts [4,5,9,10]. In contrast, ALL cells overexpress *hTERT* when compared to normal BMMNCs or to normal B or T lymphocytes [10,34]. These results were confirmed in the present series of 20 additional patients (Figure 5), where there was no significant difference in the level of DEK expression between AML, ALL, CML, CLL, and normal BM samples or normal B cells. In contrast, qChIP revealed a significantly higher DEK-*hTERT* promoter association in AML, CML, and CLL than in control cells (Figure 4). In ALL cells, which express elevated amounts of *hTERT* mRNA, the *hTERT* promoter was found depleted of DEK. A strong negative correlation linked DEK-*hTERT* promoter association and *hTERT* expression supporting an identical interplay between DEK-*hTERT* promoter association and *hTERT* expression in fresh leukemic cells and Tax-expressing cells. Knowing now that Tax represses *hTERT* in a concentration-dependent manner [6] through

the parallel recruitment of DEK on the *hTERT* promoter (present results), it could be speculated that, in AML, CML, and CLL cells, a Tax surrogate that reproduces the same telomere effects as those observed in Tax-expressing cells exists. Accordingly, deciphering the DEK interactome in leukemic cells will help identify the important factors involved in telomere-dependent chromosomal instability. Given that DEK is overexpressed in numerous tumor types, the effects of DEK on gene expression, cell differentiation, and transformation have hitherto mainly been studied through DEK overexpression and/or genetic depletion. Here, DEK was found to repress hTERT *ex vivo* in a concentration-dependent manner. However, in Tax^+ cells as in AML, CML, and CLL cells, our results did not support any correlation between the cellular amounts of DEK and the expression level of its target genes. Rather, our study pinpoints DEK redistribution along the chromatin as the main process governing its target gene transcription. Consistent with the known relationships between DEK acetylation or phosphorylation and its DNA affinity [16], the present results strongly suggest that through influencing DEK-DNA affinity and thereby target gene expression, qualitative and quantitative posttranslational changes of the DEK protein play an important role in tumorigenesis.

In conclusion, DEK was found here to repress *hTERT* through its redistribution on the *hTERT* promoter, thus permitting to explain the recently shown suboptimal *hTERT* expression in CLL [9], CML [4,5], and AML [10,34] as in certain Tax-expressing cells, such as activated HTLV-1–positive but untransformed $CD4^+$ T cells [8]. Preventing telomere elongation in proliferating premalignant cells is assumed to promote genetic instability leading to tumor initiation. In overt transformed cells, such telomere defects are assumed to sustain genetic plasticity, permitting immune escape, resistance to treatment, and relapse. DEK redistribution appears to be involved in more than 40% of Tax-targeted genes and helps explain the transcriptional pleiotropic effect of Tax, notably in the field of oncogenesis (Table 2 and Figure 6*B*). Whether DEK redistribution leads to such pleiotropic transcriptional effects in other HTLV-1–unrelated leukemias remains to be investigated.

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