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Cancer Therapy: Preclinical

Clinical Cancer Research

The Oncogene DEK Promotes Leukemic Cell Survival and Is Downregulated by both Nutlin-3 and Chlorambucil in B-Chronic Lymphocytic Leukemic Cells

Paola Secchiero[1], Rebecca Voltan[1], Maria Grazia di Iasio[1], Elisabetta Melloni[1], Mario Tiribelli[2], and Giorgio Zauli[1]

Abstract

Purpose: To characterize the role of the oncogene *DEK* in modulating the response to either Nutlin-3, a small-molecule inhibitor of the MDM2/p53 interaction, or chlorambucil in primary B-chronic lymphocytic leukemia (B-CLL) cells.

Experimental Design: DEK mRNA and protein levels were evaluated in primary B-CLL samples (n = 21), $p53^{wild\text{-}type}$ SKW6.4, $p53^{mutated}$ BJAB lymphoblastoid cell lines, and normal $CD19^+$ B lymphocytes–treated Nutlin-3 or chlorambucil (10 μmol/L, each). Knocking down experiments with either p53 or DEK small interfering RNA (siRNA) were done to investigate the potential role of p53 in controlling the expression of DEK and the role of DEK in leukemic cell survival/apoptosis.

Results: Both Nutlin-3 and chlorambucil downregulated DEK in primary B-CLL samples (n = 21) and SKW6.4 but not in BJAB cells. Knocking down p53 attenuated the effect of Nutlin-3 on DEK expression, whereas knocking down DEK significantly increased both spontaneous and Nutlin-3–induced apoptosis. Conversely, counteracting DEK downmodulation by using p53 small interfering RNA reduced Nutlin-3–mediated apoptosis. On the other hand, Nutlin-3 potently induced p53 accumulation, but it did not affect DEK levels in normal $CD19^+$ B lymphocytes.

Conclusions: These data show that the downregulation of DEK in response to either Nutlin-3 or chlorambucil represents an important molecular determinant in the cytotoxic response of leukemic cells, and suggest that strategies aimed to downregulate DEK might improve the therapeutic potential of these drugs. *Clin Cancer Res; 16(6); 1824–33. ©2010 AACR.*

Human DEK is a 375 amino acid (43 kDa) abundant nuclear protein with important functions in the architectural regulation of chromatin assembly (1, 2). It was originally discovered as part of a fusion protein discovered as the target of a chromosomal translocation event [t(6;9)(p23;q34)] in a subset of acute myeloid leukemias (3, 4), whereas only one previous study reported altered expression of DEK in a subset of B-chronic lymphocytic leukemia (B-CLL; ref. 5). It has also been reported that the expression of DEK is increased in bladder cancer, hepatocellular carcinoma, glioblastoma, melanoma, uterine cervical cancers, and ovarian cancers (6–8). Several studies have implicated DEK in a variety of cellular processes, such as DNA replication, splice site recognition, and gene transcription, as well as in the control of cell viability, differentiation, and cell-to-cell signaling (9–20).

Authors' Affiliations: [1]Department of Morphology and Embryology, University of Ferrara, Ferrara, Italy and [2]Department of Medical and Morphological Research, Division of Hematology and Bone Marrow Transplantation, University Hospital, Udine, Italy

Corresponding Author: Giorgio Zauli, Department of Morphology and Embryology, University of Ferrara, Via Fossato di Mortara 66, 44100 Ferrara, Italy. Phone: 39-0532-455579; Fax: 39-0532-207351; E-mail: giorgio.zauli@unife.it.

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Although the mechanisms through which DEK mediates its oncogenic effects are only partially understood, it has been proposed that the pro-oncogenic role of *DEK* is mediated by its ability to destabilize p53 protein and to inhibit p53 activity and p53-mediated apoptosis (12, 20). On the other hand, a recent study carried out on a series of melanoma cell lines showed that DEK exerted its oncogenic activity through the p53-independent transcriptional activation of the antiapoptotic Bcl-2 family member Mcl-1 (21).

Taking into consideration of the lack of data about the modulation of DEK in B-CLL in response to therapeutically relevant drugs, the aim of this study was to investigate the role of DEK in the cytotoxic response to either chemotherapeutic drugs or nongenotoxic activators of the p53 pathway. For this purpose, primary B-CLL samples as well as $p53^{wild\text{-}type}$ and $p53^{mutated}$ B lymphoblastoid cell lines were treated *in vitro* with either chlorambucil, which represented the treatment of choice for B-CLL for several decades (22, 23), or Nutlin-3, which showed promising cytotoxic activity against B-CLL (24–28).

Materials and Methods

Primary B-CLL cells and continuous cell lines. Peripheral blood samples were collected in heparin-coated tubes

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Translational Relevance

The authors evaluate the effect of the small-molecule Nutlin-3 and chlorambucil on the level of expression of oncogenic DEK in primary B-chronic lymphocytic leukemia cells (B-CLL) *in vitro*. These experiments confirm that Nutlin-3 induces cell death in p53-wild-type leukemic cells, which represent >85% of B-CLL at diagnosis, and allow to dissect the role of DEK in modulating leukemic cell survival in response to either Nutlin-3 or chlorambucil. Given the lack of toxic effects of Nutlin-3 in normal cells and in initial human testing, the results of this study provide insight for the design of potential clinical testing of Nutlin-3 in B-CLL.

from 21 B-CLL patients (Table 1) and from 4 healthy blood donors following informed consent, in accordance with the Declaration of Helsinki and in agreement with institutional guidelines (University-Hospital of Udine). All patients had been without prior therapy at least for 3 mo before blood collection. B-CLL samples were characterized for IgVH and p53 mutational status, ZAP-70 expression levels, and by interphase fluorescence *in situ* hybridization, as previously described (29). For $CD19^+$ B-cell purification, T lymphocytes, natural killer lymphocytes, granulocytes, and monocytes were negatively depleted from total peripheral blood mononuclear cells with immunomagnetic microbeads (MACS microbeads, Miltenyi Biotech), with a purity of >95% of resulting $CD19^+$ B-cell population. The $p53^{wild\text{-}type}$ SKW6.4 and the $p53^{mutated}$ BJAB B lymphoblastoid cell lines were purchased from the American Type Culture Collection. Cells were cultured in RPMI 1640 (Life Technologies Bethesda Research Laboratories) containing 10% fetal bovine serum, L-glutamine, and penicillin/streptomycin (Life Technologies Bethesda Research Laboratories).

Culture treatments and assessment of cell viability and apoptosis. Both primary B-CLL and lymphoblastoid cell lines were seeded at a density of 1×10^6 cells/mL before treatment with either Nutlin-3 (10 μmol/L; Cayman Chemical) or chlorambucil (10 μmol/L; Sigma Aldrich). Cell viability was examined at different time points after treatment by trypan blue dye exclusion, whereas the induction of apoptosis was quantified by Annexin V-FITC/propidium iodide staining (Immunotech) followed by flow cytometry analysis, as previously detailed (30).

RNA analyses. Total RNA was extracted from cells by using the Qiagen RNeasy mini-kit (Qiagen) according to the supplier's instructions. The quality of the RNA

Table 1. Clinical and laboratory features of patients with CLL

Patient no.	Sex	Age, y	Rai stage	DT*	FISH†	ZAP-70‡
1	F	80	1	28	+12	High
2	F	74	2	6	13q–, +12	Low
3	M	71	0	16	17p–, +12	High
4	M	67	4	>24	ND	Low
5	F	55	0	10	11q–	High
6	M	57	1	60	ND	Low
7	M	62	0	>72	11q–	Low
8	M	64	1	14	13q–	High
9	M	51	0	37	Nor	Low
10	M	59	2	28	13q–	Low
11	M	61	2	3	17p–	ND
12	M	62	4	18	ND	Low
13	F	71	2	28	Nor	High
14	M	69	2	19	13q–; 17p–	Low
15	M	62	4	6	17p–	High
16	F	79	3	16	12+	Low
17	F	60	1	28	Nor	Low
18	M	73	1	16	Nor	Low
19	M	86	3	19	13q–	ND
20	F	64	0	ND	Nor	High
21	M	68	0	14	ND	Low

Abbreviations: F, female; M, male; ND, not done.
*Doubling time: months.
†FISH defects: Nor, normal cytogenetic; negative (–), deletion; positive (+), trisomy.
‡ZAP-70 expression was determined by Western blot analysis.

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preparations was verified by agarose gel and, when necessary, further purification was done with the RNeasy clean-up system (Qiagen) to remove chromatin DNA. Total RNA was transcribed into cDNA, using the AMV Reverse transcriptase (Finnzyme). Modulation of the DEK and MDM2 gene expression upon Nutlin-3 treatment was assessed with the Real Time Thermal Analyzer Rotor-Gene 6000 (Corbett) by SYBR Green real-time PCR detection

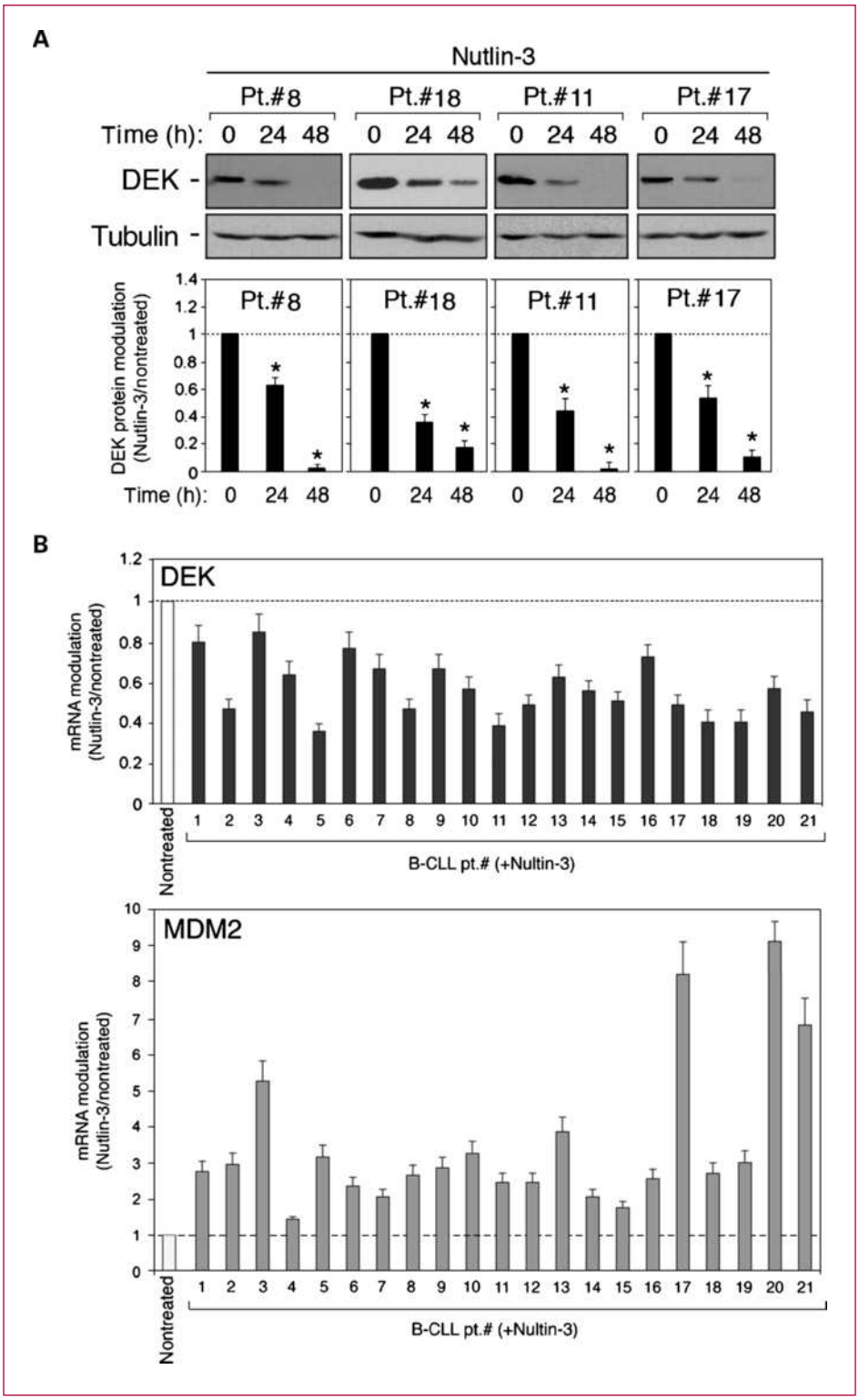

Fig. 1. Modulation of DEK by Nutlin-3 in primary B-CLL samples. After exposure to Nutlin-3 (10 μmol/L), B-CLL samples were analyzed for DEK protein (A) and mRNA (B). A, DEK protein levels, analyzed by Western blot, are shown for representative B-CLL patient samples. Tubulin staining is shown as loading control. Protein bands were quantified by densitometry and level of DEK was calculated for each time point after normalization to tubulin in the same sample. Unstimulated basal expression was set as unity (hatched line). Columns, mean of determinations each done in triplicate; bars, SD. *, $P < 0.05$ with respect to nontreated cultures (time 0). B, levels of DEK and MDM2 mRNA were analyzed by quantitative RT-PCR. Results are expressed as fold of DEK and MDM2 modulation by Nutlin-3, after 48 h of treatment, with respect to the control nontreated cultures set to 1 (hatched line). Columns, mean of results from experiments each done in triplicate; bars, SD.

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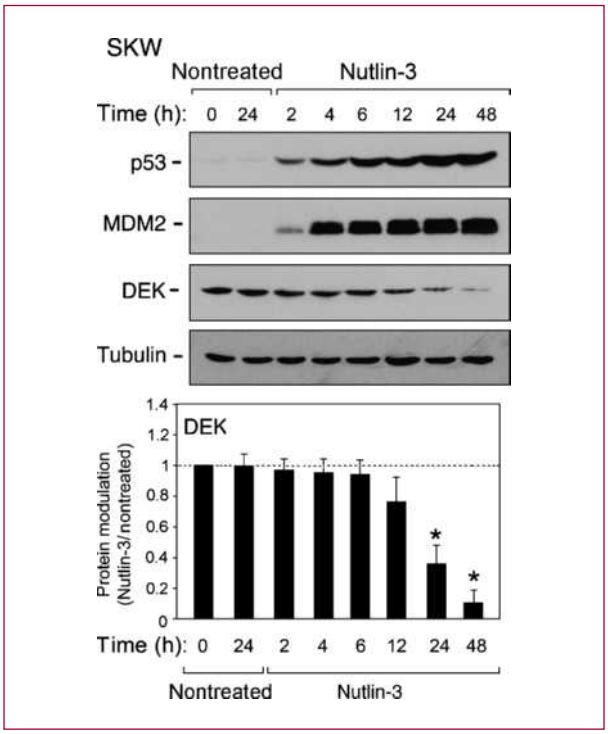

Fig. 2. Effect of Nutlin-3 on DEK expression in the $p53^{wild\text{-}type}$ SKW6.4 B lymphoblastoid cell line. SKW6.4 cell line was either left nontreated or exposed to Nutlin-3 (10 μmol/L). Levels of p53, MDM2, and DEK proteins were simultaneously assessed by Western blot analysis in cell lysates harvested at the indicated time points. Tubulin staining is shown as a loading control. Representative examples of Western blot results of four independent experiments are shown. Protein bands were quantified by densitometry and level of DEK was calculated for each time point after normalization to tubulin in the same sample. Results are expressed as DEK protein modulation by Nutlin-3 with respect to the control nontreated cultures set to 1 (hatched line). Columns, mean of three independent experiments; bars, SD. *, $P < 0.05$ with respect to nontreated cultures.

method, using the SABioscience's RT^2 Real-Time Gene Expression Assays, which include specific validated primer sets and PCR master mixes (SABioscience Corp.). All samples were run in triplicates.

Western blot analyses. Cells were lysed in ice-cold RIPA buffer [50 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 0.1% SDS, 1% Nonidet P-40, 0.25% sodium desoxycholate] supplemented with protease inhibitors (ROCHE) on ice for 1 h. Cell lysates were passed through 27G needles, were added with a loading buffer [250 mmol/L Tris (pH 6.8), 2% SDS, 40% Glycerin, 20% β-mercaptoethanol], and boiled for 2 min. Because DEK protein is predominantly found associated to the nuclear fraction (1), we cannot completely rule out the possibility that part of nuclear DEK protein is lost with the procedure adopted in our study. Equal amounts of protein for each sample were migrated in acrylamide gels and blotted onto nitrocellulose filters, as previously described (31). The following monoclonal antibody were used in our experiments: anti p53 (DO-1), anti-MDM2 (both purchased from Santa Cruz Biotechnology), anti-DEK (BD Transduction Laboratories), and anti-tubulin (Sigma-Aldrich). After incubation with peroxidase-conjugated anti-mouse IgG, specific reactions were revealed with the enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech) and densitometry values were estimated by the ImageQuant TL software (Amersham).

Transfection experiments. Cells (1.25×10^6) were resuspended into 0.1 mL of Nucleofector solution V of human nucleofector kit V (Amaxa). Two micrograms of plasmid DNA (green fluorescent protein construct) or 1 μg of small interfering RNA (siRNA) were mixed with the 0.1 mL of cell suspension, transferred into a 2.0-mm electroporation cuvette, and nucleofected using an Amaxa Nucleofector II apparatus, following the manufacturer's guidelines. After transfection, cells were immediately transferred into complete medium and cultured in six-well plates at 37°C. Transfection efficiency was estimated in each experiment by scoring the number of green fluorescent protein–positive cells by flow cytometry analysis. SiRNA were designed and manufactured by Ambion, Inc., according to the current guidelines for effective gene knockdown by this method. Based on preliminary validation experiments, siRNA selected were as follows: target #1 for *TP53* 5′-GGGAGUUGUCAAGUCUUGCtt-3′ (sense) and 5′-GCAAGACUUGACAACUCCCtc-3′ (antisense); target #2 for *TP53*, 5′-GGGUUAGUUUACAAUCAGCtt-3′ (sense) and 5′-GCUGAUUGUAAACUAACCCtt-3′ (antisense); target #1 for *DEK*, 5′-GGUGUGCACUGUGAGAUCAtt-3′ (sense) and 5′-UGAUCUCACAGUGCACACCct-3′ (antisense); target #2 for *DEK*, 5′-GGACUGUGCGGAGCAUGUAtt-3′ (sense) and 5′-UACAUGCUCCGCACAGUCCag-3′ (antisense); and target #3 for *DEK*, 5′-UCGUCUACCUGGAGAUUGAtt-3′ (sense) and 5′-UCAAUCUCCAGGUAGACGAtg-3′ (antisense). A cocktail of three different negative control siRNAs, each composed of a 19 bp-scrambled sequence with 3′ dT overhangs (Ambion's *Silencer* negative control siRNA), was used to show that the transfection did not induce nonspecific effects on gene expression.

Statistical analysis. The results were evaluated by using ANOVA with subsequent comparisons by Student's *t* test and with the Mann-Whitney rank-sum test. Statistical significance was defined as $P < 0.05$.

Results

Nutlin-3 downregulates DEK in primary B-CLL cells. Primary leukemic cells, obtained from 21 B-CLL patients and characterized for Rai stage, ZAP70 levels, and fluorescence *in situ* hybridization analysis (Table 1), were treated for 24 to 48 hours with Nutlin-3 (10 μmol/L), a nongenotoxic activator of the p53 pathway (32). Nutlin-3 significantly downmodulated the amount of the DEK protein, assessed by Western blot followed by densitometric analysis (Fig. 1A), as well the steady-state mRNA levels of DEK, analyzed by quantitative reverse transcription-PCR (RT-PCR; mean ± SD, 0.55 ± 0.14; fold of decrease, $P < 0.01$; Fig. 1B). On the other hand, Nutlin-3 potently

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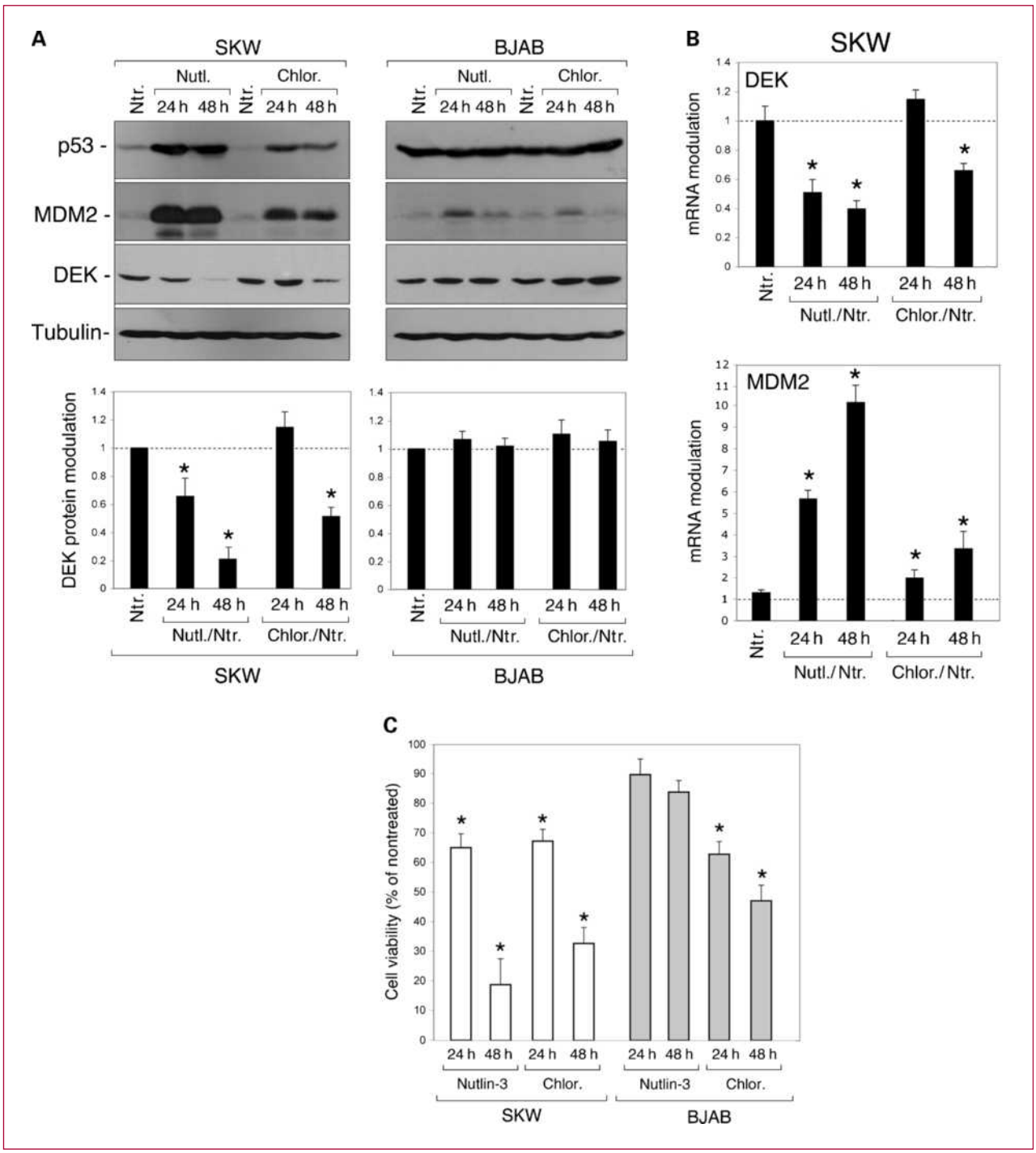

Fig. 3. Comparative analysis of the effects of Nutlin-3 and chlorambucil in $p53^{wild\text{-}type}$ and in $p53^{mutated}$ B lymphoblastoid cell lines. The $p53^{wild\text{-}type}$ SKW6.4 and the $p53^{mutated}$ BJA B-cell lines were either left nontreated (Ntr.) or exposed to Nutlin-3 (Nutl.) or chlorambucil (Chlor.), both used at 10 μmol/L. A, levels of p53, MDM2, and DEK proteins were simultaneously assessed by Western blot analysis in cell lysates harvested at the indicated time points. Tubulin staining is shown as a loading control. Representative examples of Western blot results of three independent experiments are shown. Protein bands were quantified by densitometry, and DEK levels were calculated for each time point after normalization to tubulin in the same sample. Results are expressed as DEK protein modulation by either Nutlin-3 or chlorambucil with respect to the control nontreated cultures. Columns, mean of three independent experiments; bars, SD. *, $P < 0.05$ with respect to nontreated cultures. B, levels of DEK and MDM2 mRNA were analyzed by quantitative RT-PCR. Results are expressed as fold of DEK and MDM2 modulation in Nutlin-3– or chlorambucil-treated cultures with respect to the control nontreated cultures set to 1 (hatched line). Columns, mean of results from experiments each done in triplicate; bars, SD. *, $P < 0.05$ with respect to nontreated cultures. C, data of cell culture viability upon the indicated treatments are presented; columns, mean of five independent experiments each done in duplicate. *, $P < 0.05$ with respect to nontreated cultures (set to 100).

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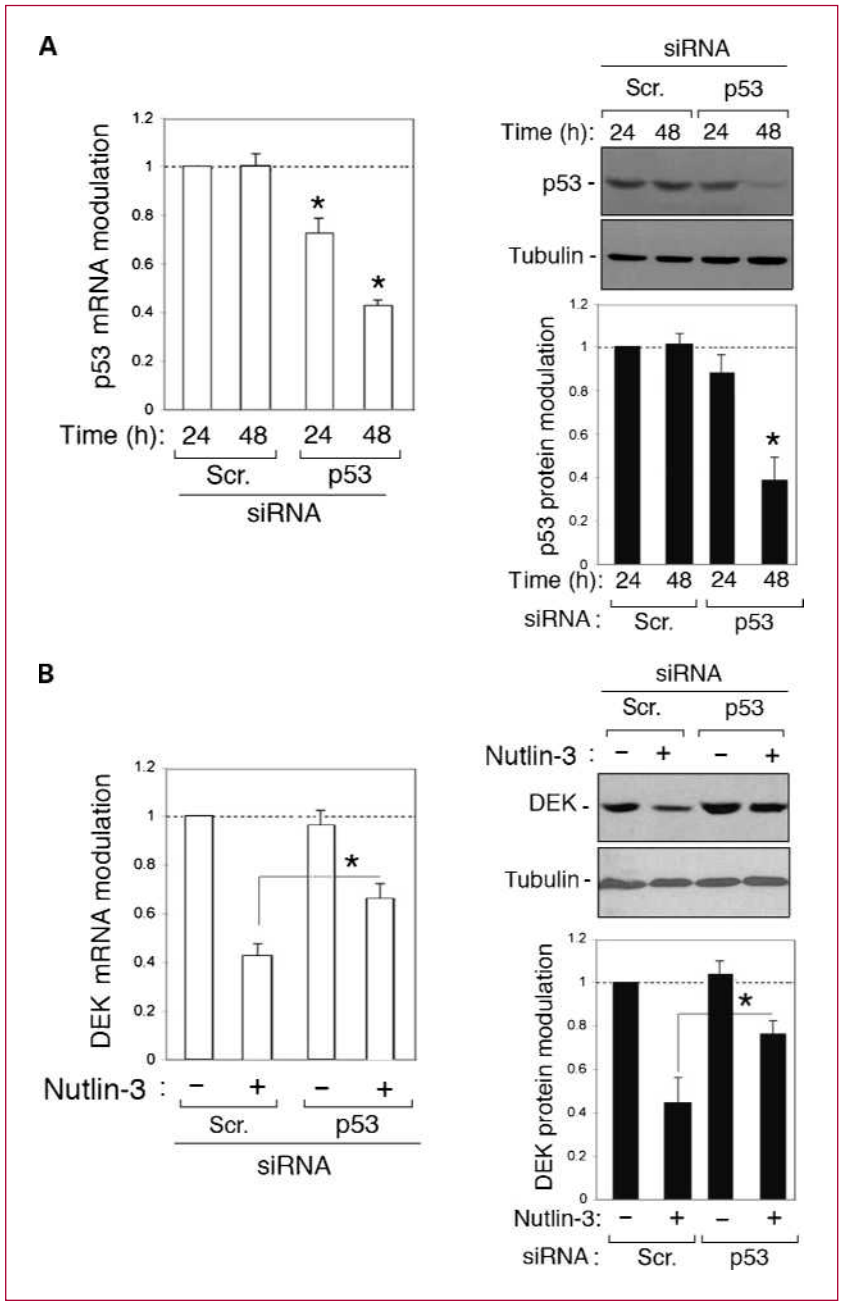

Fig. 4. Effect of p53 silencing on the ability of Nutlin-3 to downregulate DEK in $p53^{wild\text{-}type}$ B lymphoblastoid cells. SKW6.4 cells were transfected with either control scrambled (scr.) siRNA or p53 siRNA before treatment with Nutlin-3 for 48 h. After transfection, levels of p53 (A) and DEK (B) mRNA and proteins were assessed by quantitative RT-PCR and Western blot analysis. RT-PCR results are expressed as fold of p53 and DEK modulation with respect to the scrambled control transfected cultures. Representative examples of Western blot results of three independent experiments are shown; tubulin staining is shown as loading control. Protein bands were quantified by densitometry and levels of p53 and DEK were calculated for each time point after normalization to tubulin in the same sample. Results are expressed as p53 or DEK protein modulation with respect to the control scrambled-transfected cultures set to 1 (hatched line). Columns, mean of three independent experiments; bars, SD. *, $P < 0.05$.

upregulated the steady-state mRNA levels of MDM2 (mean ± SD, 3.43 ± 2.04; fold of increase, $P < 0.01$; Fig. 1B), which represents a major transcriptional target of p53 (33). In parallel, a small subset of B-CLL samples ($n = 5$) was treated with chlorambucil (10 μmol/L), which induced a cytotoxicity comparable with Nutlin-3. In addition, chlorambucil downregulated DEK at both the protein and mRNA levels (data not shown).

Nutlin-3 and chlorambucil selectively downregulate DEK expression in $p53^{wild\text{-}type}$ but not in $p53^{mutated}$ B lymphoblastoid cell lines. To ascertain whether DEK downmodulation might involve p53, the effect of Nutlin-3 or chlorambucil was next analyzed on the levels of DEK protein as well as of p53 and MDM2 proteins in $p53^{wild\text{-}type}$ and $p53^{mutated}$ B lymphoblastoid cell lines. Nutlin-3 induced the rapid (from 2 hours onwards) and progressive accumulation of both p53 and MDM2 proteins in the $p53^{wild\text{-}type}$ SKW6.4 cells (Fig. 2). Conversely, the levels of DEK protein showed a progressive and significant ($P < 0.05$) decline, starting 24 hours after Nutlin-3 treatment (Fig. 2).

When analyzed comparatively, we observed that chlorambucil was less potent than Nutlin-3 in promoting the accumulation of both p53 and MDM2 proteins, as well as in inducing the downregulation of DEK protein

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(Fig. 3A). On the contrary, in the p53^{mutated} BJAB cells, characterized by constitutive high basal levels of p53, neither Nutlin-3 nor chlorambucil induced p53 and MDM2 protein accumulation and did not affect DEK protein levels (Fig. 3A). Consistent with the results obtained at the protein level, exposure of SKW6.4 cells to either Nutlin-3 or chlorambucil downregulated the steady-state mRNA levels of DEK, while increasing the steady-state mRNA levels of MDM2 (Fig. 3B). Of note, the ability of Nutlin-3 to activate the p53 pathway fully correlated with its cytotoxic activity, as shown by the progressive and significant ($P < 0.05$) decrease of cell viability, observed at 24 and 48 hours after Nutlin-3 treatment in p53$^{wild\text{-}type}$ but not in the p53^{mutated} cell lines (Fig. 3C). On the other hand, chlorambucil was less potent than Nutlin-3 in downregulating DEK, an event occurring only after treatment of SKW6.4 cells for 48 hours (Fig. 3A-B), as well as in inducing p53 and its transcriptional target MDM2 (Fig. 3A-B). However, in spite of its lower efficacy in activating the p53 pathway and in downregulating DEK in SKW6.4 cells, chlorambucil exhibited cytotoxic activity against SKW6.4 comparable with that of Nutlin-3 (Fig. 3C). In addition, at variance to Nutlin-3, chlorambucil significantly ($P < 0.05$) reduced cell viability also in p53^{mutated} BJAB cells (Fig. 3C), clearly suggesting that chlorambucil promotes leukemic cytotoxicity also through p53- and DEK-independent pathways.

Knocking down p53 partially counteracts the Nutlin-3–mediated ability to downregulate DEK. To further investigate whether the Nutlin-3–mediated downregulation of

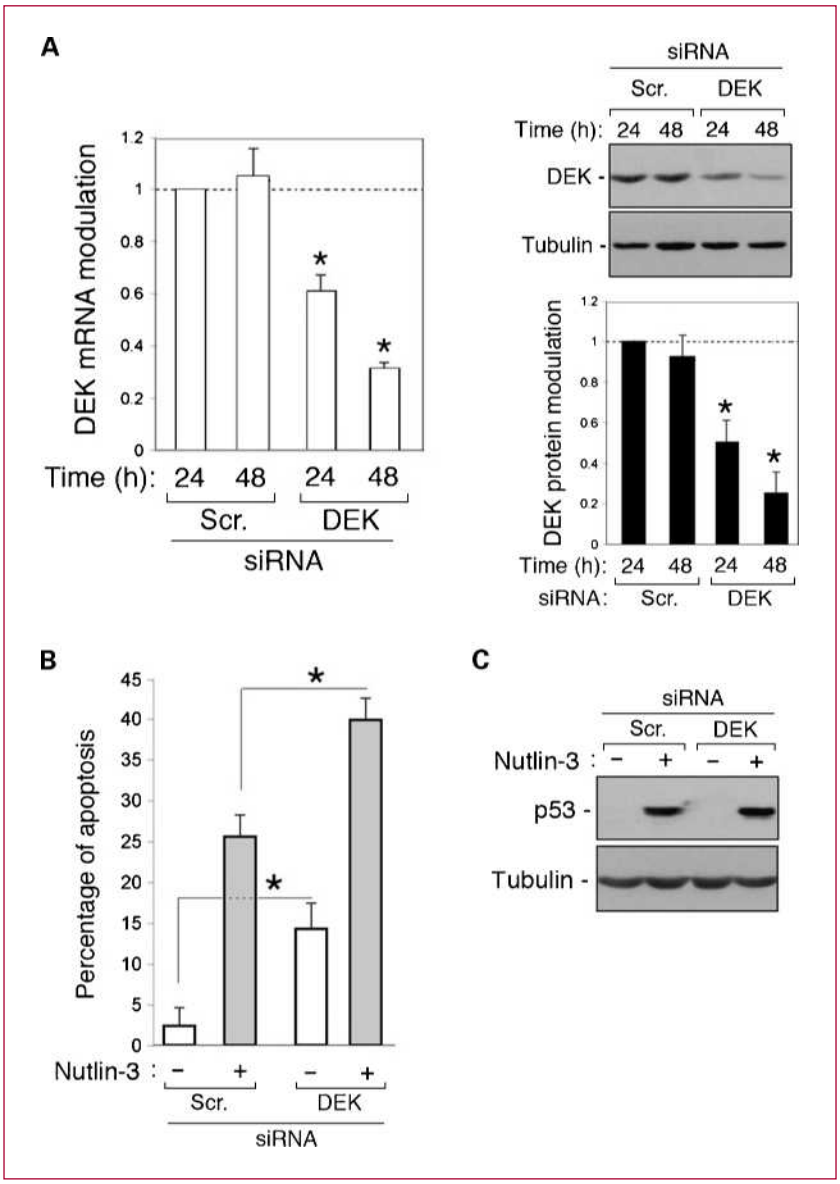

Fig. 5. Effects of DEK downmodulation on Nutlin-3–mediated cytotoxicity in p53$^{wild\text{-}type}$ lymphoblastoid cell line. SKW6.4 cells were transfected with either control scrambled (scr.) siRNA or DEK siRNA before treatment with Nutlin-3 for 48 h. After transfection, levels of DEK mRNA and protein were assessed by quantitative RT-PCR and Western blot followed by densitometric analyses (A). Results were expressed as fold of DEK modulation with respect to the control scrambled-transfected cultures set to 1 (hatched line). B and C, cultures transfected with either control scrambled siRNA or DEK siRNA were analyzed for apoptosis induction (B) and p53 accumulation (C) upon treatment with Nutlin-3. Columns, mean of results from three independent experiments; bars, SD. *, $P < 0.05$. C, representative examples of Western blot results of three independent experiments are shown; tubulin staining is shown as loading control.

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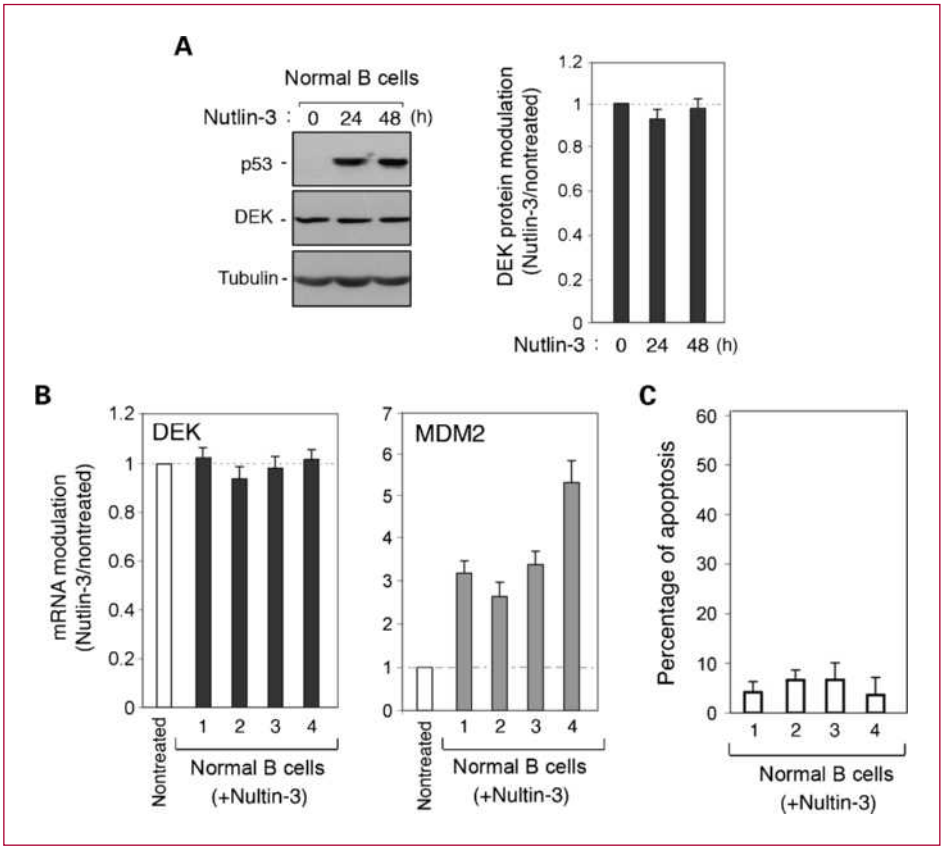

Fig. 6. Lack of DEK down-modulation by Nutlin-3 in normal CD19$^+$ B cells. After exposure to Nutlin-3, samples from normal CD19$^+$ B cells were analyzed for DEK protein (A) and mRNA levels (B). A, Western blot analysis of DEK protein levels, as well as of p53 accumulation upon Nutlin-3 treatment, is shown for a representative CD19$^+$ B-cell culture. Tubulin staining is shown as loading control. B, levels of DEK and MDM2 mRNA were analyzed by quantitative RT-PCR. After normalization to the level of glyceraldehyde-3-phosphate dehydrogenase mRNA, results were expressed as fold of DEK and MDM2 modulation in Nutlin-3–treated cultures with respect to the control nontreated cultures. C, normal CD19$^+$ B-cell cultures were analyzed for apoptosis upon 48 h of treatment with Nutlin-3. Columns, mean of results from experiments each done in triplicate; bars, SD.

DEK requires a functional p53 pathway, in the next experiments, we have used predetermined optimal experimental conditions to specifically knock down *TP53* gene expression by siRNA transfection of SKW6.4 cells (Fig. 4A). Taking into consideration that in our experimental conditions the transfection efficiency was approximately 40% to 50%, it is noteworthy that knocking down p53 partially, but significantly ($P < 0.05$), counteracted the ability of Nutlin-3 to downregulate DEK expression (Fig. 4B).

Knocking down of DEK increases both the spontaneous and Nutlin-3–induced apoptosis. Because a previous study suggesting that DEK confers resistance to apoptosis induced by genotoxic stress in solid tumors (34), it was of crucial interest to investigate whether DEK plays a role in modulating cell survival/apoptosis in response to Nutlin-3 or chlorambucil. For this purpose, after having obtained a significant downmodulation of DEK expression after 48 hours of transfection with DEK siRNA, as documented by quantitative RT-PCR and by Western blot (Fig. 5A), cells were either left nontreated or exposed to Nutlin-3 before analyzing the amount of apoptosis. In SKW6.4 cells transfected with DEK-specific siRNAs, both the spontaneous (white columns) and Nutlin-3–mediated (gray columns) cytotoxicity were significantly ($P < 0.05$) increased with respect to cells transfected with scrambled control siRNA (Fig. 5B). On the other hand, transfection with p53 siRNA significantly counteracted (from 25.5 ± 3 to 11 ± 2.5, $P < 0.05$) the ability of Nutlin-3 to induce cell death, consistently with the effect of hampering Nutlin-3–mediated downregulation of DEK (Fig. 4B).

Because DEK overexpression has been shown previously to mediate antiapoptotic effects through destabilization of p53 protein and inhibition of p53 activity (12, 20), we have also analyzed the potential effect of DEK siRNA on p53 levels. As shown in Fig. 5C, the accumulation of p53 induced by Nutlin-3 in SKW6.4 cells was not affected by DEK downmodulation (Fig. 5C).

Nutlin-3 does not downregulate DEK in primary normal CD19$^+$ B lymphocytes. In the last group of experiments, we have investigated the effect of Nutlin-3 on DEK expression in primary normal CD19$^+$ B lymphocytes, purified from normal blood donors. As shown in Fig. 6, Nutlin-3 potently induced p53 accumulation in primary CD19$^+$ B lymphocytes but, in striking contrast to what observed in primary B-CLL cells and leukemic cell lines, it did not induce DEK downregulation either at the protein (Fig. 6A) or mRNA (Fig. 6B) level, indicating that p53 activation in nontransformed primary cells was insufficient to induce DEK downregulation. Moreover, in keeping with an important role of DEK in promoting cell survival, Nutlin-3 did not exhibit cytotoxic activity in primary normal B-cell cultures, compared with p53$^{wild\text{-}type}$ leukemic cell lines or primary B-CLL cells (Fig. 6C).

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Discussion

In response to a variety of stimuli, such as cellular stress induced by chemotherapeutic drugs, the p53-MDM2 interaction is disrupted and p53 rapidly accumulates within the cell (32). Alternatively, p53 can accumulate in response to selective small-molecule inhibitors of the p53-MDM2 interaction, which binds MDM2 in the p53 binding pocket with high selectivity and can release p53 from negative control leading to effective stabilization of p53 and activation of the p53 pathway (35). In this study, we have shown for the first time that both chemotherapeutic drugs (chlorambucil) and nongenotoxic activators of the p53 pathway (Nutlin-3) significantly downregulated DEK in primary $p53^{wild\text{-}type}$ B-CLL and lymphoblastoid SKW6.4 cells but not in $p53^{mutated}$ (BJAB) B lymphoblastoid cells. Although these data clearly indicate that a $p53^{wild\text{-}type}$ status is necessary to observe the DEK downregulation in response to either Nutlin-3 or chlorambucil in leukemic cells, the exact role of p53 in DEK regulation remains to be determined. In fact, by using bioinformatic programs (TESS[3] and TFSEARCH)[4], no p53 consensus sequences were found in the DEK promoter. Moreover, whereas knocking down p53 partially counteracted the ability of chlorambucil or Nutlin-3 to downregulate DEK in leukemic cells, Nutlin-3 potently upregulated p53 accumulation in primary $CD19^+$ cells without inducing DEK downregulation. Therefore, although $p53^{wild\text{-}type}$ status seems to be necessary, but not sufficient for the Nutlin-3–mediated downmodulation of DEK, further work is needed to clearly establish the role of additional transcription factors in mediating the downregulation of DEK by either Nutlin-3 or chemotherapeutic drugs.

The major conclusion of our study is that DEK plays an important role in promoting the survival/counteracting apoptosis in lymphoblastoid B cells. This was shown by the experiments done with siRNA specific for *DEK*. In fact, knocking down DEK resulted in a significant increase of both spontaneous and Nutlin-3– or chlorambucil-induced apoptosis in SKW6.4 cells, thus suggesting that the downregulation of DEK is an important molecular mechanism involved in the proapoptotic activity of both chemotherapeutic drugs and novel activators of the p53 pathway in leukemic cells. Thus, although it has been argued that transcriptional independent mechanisms play a key role in mediating the cytotoxic activity of p53 (36, 37), perhaps due to the presence of negative feedback loops (38), our data show for the first time that the transcriptional downregulation of the oncogene *DEK* represents an important additional mechanism of action by which Nutlin-3 promotes apoptosis in leukemic cells. However, although previous studies have proposed that the overexpression of DEK inhibits the transcriptional activity of p53 (12, 20), silencing of *DEK* expression by siRNA in SKW6.4 lymphoblastoid cells did not induce any accumulation of p53 protein, nor of p53 target genes, such as MDM2, in response to either Nutlin-3 or chlorambucil. In keeping with a recent study done on melanoma cell lines, showing that siRNA specific for *DEK* did not change the levels of p53 in the absence or presence of doxorubicin (21), the contribution of DEK to promote tumor cell survival is likely due to its ability to upregulate antiapoptotic genes of the *Bcl-2* family (21), rather than to directly control the p53 pathway.

In conclusion, we have shown for the first time that the activation of p53 represses the transcription of *DEK* in $p53^{wild\text{-}type}$ B-CLL cells and we propose that such downregulation is an important mediator of Nutlin-3 and chlorambucil cytotoxic activity. The relevance of our findings is underscored by the fact that, in contrast to most solid tumors, p53 is mutated in approximately 10% to 15% of both myeloid and lymphoid leukemias at diagnosis (39). Therapeutic strategies able to downregulate DEK should be further explored to improve the antileukemic activity of both conventional and novel antileukemic drugs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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[3] http://www.cbil.upenn.edu/cgi-bin/tess/tess?RQ=WELCOME
[4] http://www.cbrc.jp/research/db/TFSEARCH.html

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Paola Secchiero, Rebecca Voltan, Maria Grazia di Iasio, et al.

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