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Downregulation of Telomerase in $CoCl_2$-Induced Apoptosis of Myeloid Leukemic Cells

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Abstract: Many reports showed that hypoxia-inducible factor-1α (HIF-1α), the main factor activated by hypoxia, plays a role in the transcriptional regulation of the human telomerase reverse transcriptase (hTERT), one of the critical elements of the oncogenic process. As a hypoxia-mimetic agent, cobalt chloride ($CoCl_2$) can induce the accumulation of HIF-1α. Herein, we demonstrated that hTERT expression was greatly decreased during $CoCl_2$-induced apoptosis in leukemic cell lines while overexpression of hTERT inhibited $CoCl_2$-induced apoptosis. Knockdown of HIF-1α by shRNA in U937 cells did not abrogate $CoCl_2$-induced hTERT downregulation nor $CoCl_2$-induced apoptosis while inducible expression of HIF-1α decreased the expression of hTERT. Furthermore, $CoCl_2$ could decrease the c-myc protein in all the cells tested, no matter the HIF-1α was silenced or not. Taken together, we demonstrated that downregulation of hTERT contributes to $CoCl_2$-induced apoptosis in leukemic cell lines and HIF-1α is not indispensable for $CoCl_2$ induced downregulation of hTERT.

Key Words: Telomerase, Apoptosis, $CoCl_2$, HIF-1α, Leukemia.

INTRODUCTION

Telomerase is a ribonucleoprotein enzyme complex that is responsible for the stabilization of telomere length at the chromosome ends. Human telomerase activity minimally needs the catalytic subunit known as hTERT (human telomerase reverse transcriptase) and an RNA template. hTERT is the rate-limiting subunit of telomerase [1]. Telomerase activity is lacking or undetectable in most human normal cells, but presenting in most tumor cells. Recent works suggest that telomerase can inhibit apoptosis through telomerase activity-dependent and independent manners while inhibition of telomerase expression can trigger apoptosis [2]. Moreover, downregulation of telomerase contributes to the effect of several chemotherapy drugs including arsenic trioxide and all-trans retinoid acid (ATRA) [3, 4]. These data indicate that telomerase is a relevant target for drug development in cancer therapy.

The majority of solid tumors contain regions of low oxygen tension (hypoxia). In response to hypoxia, mammalian cells undergo transcriptional activation of a series of genes involved in angiogenesis, cell proliferation and survival [5, 6]. Hypoxia-inducible factor-1 (HIF-1) is the main element that mediates such a response. Several reports have investigated the possible effects of hypoxic treatment or overexpression of HIF-1α on hTERT expression and its telomerase activity. However, controversial results were obtained [7-11]. Some observations reported that hypoxia upregulated hTERT expression and telomerase activity [7-10], whereas one report showed a significant inhibition of hTERT expression by hypoxia-induced HIF-1α [11].

Previously, several groups including ours demonstrated that cobalt chloride ($CoCl_2$), a commonly used hypoxia-mimetic agent [12], can induce apoptosis in different cell lines [13-16]. Proposed mechanisms of action for $CoCl_2$ induced apoptosis include production of reactive oxygen species (ROS), loss of mitochondrial transmembrane potentials, upregulation of Fas receptor and Fas ligand *et al*. In this work, we investigated the role of hTERT in $CoCl_2$-induced apoptosis and the relationship between $CoCl_2$-induced HIF-1α accumulation and hTERT expression. We found that downregulation of hTERT contributes to $CoCl_2$ induced apoptosis in leukemic cell lines and HIF-1α accumulation is not indispensable for $CoCl_2$ induced downregulation of hTERT.

MATERIALS AND METHODOLOGY

Reagents and cell lines. Acute myeloid leukemic cell lines, including acute monocytic leukemia cell line U937, THP-1, and acute promyelocytic leukemia (APL) cell line NB4 as well as NB4-derived ATRA-resistant NB4-LR1 cells, were used in this work. In addition, the retrovirally infected NB4-LR1/hTERT-GFP cells expressing both hTERT protein and the green fluorescent protein (GFP) reporter from the same transcript, and the NB4-LR1/GFP sub-

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line expressing only the GFP control vector cells, were established as described previously [17]. U937-shR-α14 (shR-α14) and U937-shR-NC (shR-NC) were U937 cells transfected with specific shRNA against HIF-1α and negative control shRNA, respectively [18]. $U937T^{pool}$ cells were U937T stable transformants with inducible HIF-1α expression, while $U937T^{empty}$ cells were transformants with control vector [18]. All cells were cultured at 37ºC in RPMI 1640 medium (Sigma-Aldrich, St Louis, MI) supplemented with 10% heat-inactivated fetal calf serum (Gibco BRL, Gaithersburg, ML), penicillin (100 IU/ml), streptomycin (100 μg/ml) in 5% CO_2/95% air humidified atmosphere. For the treatments, cells were seeded at a density of 3×10^5 cells/ml in the medium supplemented with or without $CoCl_2$ (Sigma-Aldrich, St Louis, MO). Cell death was determined by trypan-blue exclusion assay. For morphological studies, cells were collected onto cytocentrifuge slides (Shandon, Runcorn, UK), stained with Wright's stain, and observed through light microscopy (Olympus BX-51, Olympus Optical, Japan).

Flow cytometry analysis. To assess the distribution of DNA content, 10^6 cells were collected, rinsed and fixed overnight with 75% cold ethanol at −20ºC. After treated with 100 μg/ml RNase A in Tris-HCl buffer (pH 7.4) and stained with 25 μg/ml propidium iodide (PI, Sigma, St. Louis, MO), cells were analyzed by flow cytometry (FACSCalibur, BD Biosciences, San Diego, CA) using CellQuest Pro software (BD Biosciences). Ten thousand cells were acquired and analyzed for DNA content. The number of cells in sub-G_1 phase (apoptotic cells) was expressed as percentages of total events (10,000 cells).

Real-time quantitative RT-PCR. Total cellular RNA was extracted using TRIzol reagent (Gibco BRL, Gaithersburg, ML) and treated with DNase (Promega, Madison, WI). Reverse transcription (RT) was performed with 2 μg of mRNA by using the TaKaRa RNA PCR kit (Takara, Dalian, China) according to the manufacturer's instructions. For real-time quantitative RT-PCR, the PCR sequences specific for hTERT mRNA were 5'- CGG AAG AGT GTC TGG AGC AA-3'(sense) and 5'-CTC CCA CGA CGT AGT CCA TG-3' (antisense), and for β-actin used as an internal control, 5'-CAT CCT CAC CCT GAA GTA CCC-3'(sense), and 5'-AGC CTG GAT AGC AAC GTA CAT G-3' (antisense). PCR conditions for 40 cycles were denaturation at 95 ºC for 20 seconds, annealing at 56 ºC for 20 seconds and extension at 72 ºC for 30 seconds. Amplification cycles were preceded by a denaturation step (95 ºC for 10 minutes) and followed by an elongation step (72 ºC for 10 minutes). RT-PCR was performed using a ABI 7300 Real-Time PCR System (Perkin Elmer Applied Biosystems, USA) and a SYBR Green PCR Master Mix kit (Applied Biosystems). hTERT mRNA expression levels were calculated based on cycle threshold (C_t) values and normalized using the endogenous reference β-actin. All RT-PCR were repeated at least three times.

Western blots. Protein extracts (10 μg) were equally loaded on 8-12% SDS-polyacrylamide gel, electrophoresed, and blotted onto Immobilon-PVDF membranes (Schleicher & Schuell, Dassel, Germany). Afterwards, the membranes were blocked with 5% nonfat milk in TBS for 2 hours, then incubated overnight at 4°C with rabbit anti-hTERT (Rockland, Gilbertsville, PA), mouse anti-HIF-1α (BD Transduction, San Jose, CA), rabbit anti-c-myc (Santa Cruz, CA), rabbit anti-cleaved caspase 3, rabbit anti-caspase 8 (Cell Signaling, Beverly, MA), or mouse anti-PARP (Santa Cruz, CA). Following incubation with horseradish peroxidase (HRP)-linked secondary antibodies (Cell Signaling, Beverly, MA), the signals were detected by the chemiluminescence phototope-HRP kit (Cell Signaling, Beverly, MA). The same blots were re-probed with anti-β-actin (Merck, Darmstadt, Germany) or anti-β-tubulin (Sigma-Aldrich, St Louis, MI) antibody to ascertain equal loading of proteins among lanes.

Telomerase activity assay. Telomerase activity assay (TRAP-ELISA) was performed using the TeloTAGGG Telomerase PCR ELISA kit according to the manufacturer's instructions (Roche, Basel, Switzerland) with 1 μg of protein extracts and 30 cycles. Telomerase activity was expressed as absorbance value (OD) measured using a microtiter reader at 450 nm with a reference wavelength of 630 nm.

Statistical Analysis. The Student's t-test was used to compare the difference between two different groups. A value of $P<0.05$ was considered to be statistically significant.

RESULTS

HIGHER CONCENTRATION OF $COCL_2$ INDUCES APOPTOSIS OF LEUKEMIC CELL LINES

As shown previously [13], $CoCl_2$ at high concentration (200 μM) decreased cell viability (Fig. **1A**) and induced apoptosis in leukemic NB4 and U937 cell lines. The similar apoptosis induction could also be seen in two other leukemic cell lines, the THP-1 and NB4-LR1 cells, as revealed by cell viability (Fig. **1A**), the appearance of typical morphological features [such as cell shrinking, chromatin condensation and nuclear fragmentation with intact cell membrane (Fig. **1B** and data not shown)], appearance of hypoploid cells (cells in sub-G_1) (Fig. **1C**), activation of caspase 3 and caspase 8 and degradation of PARP, a specific substrate of caspase 3 (Fig. **1D**).

APOPTOSIS-INDUCING CONCENTRATION OF $COCL_2$ DOWNREGULATES HTERT EXPTESSION IN LEUKEMIC CELL LINES

As expected, 200μM of $CoCl_2$ induced the accumulation of HIF-1α protein in all four leukemic cells tested (Fig. **2A**). Because HIF-1α has been implicated in the regulation of hTERT in many tumor cell lines [7-9, 11, 19], we next examined whether $CoCl_2$ treatment could affect hTERT expression and activity. The results showed that, compared with the untreated cells, $CoCl_2$ treatment induced a great reduction in hTERT mRNA content in these leukemic cell lines, as examined by real-time quantitative PCR (Fig. **2B**). Furthermore, hTERT protein also decreased in $CoCl_2$-treated cells (Fig. **2A**) although not as significant as hTERT transcripts. We extrapolated that this might be due to the long half-life of the hTERT protein [20]. In line with this, telomerase activity was not significantly modified in these $CoCl_2$-treated cells (Fig. **2C**). Besides, telomerase activity is not always strictly related to the transcription of the catalytic subunit [3]. In addition, we also tested whether $CoCl_2$ is able

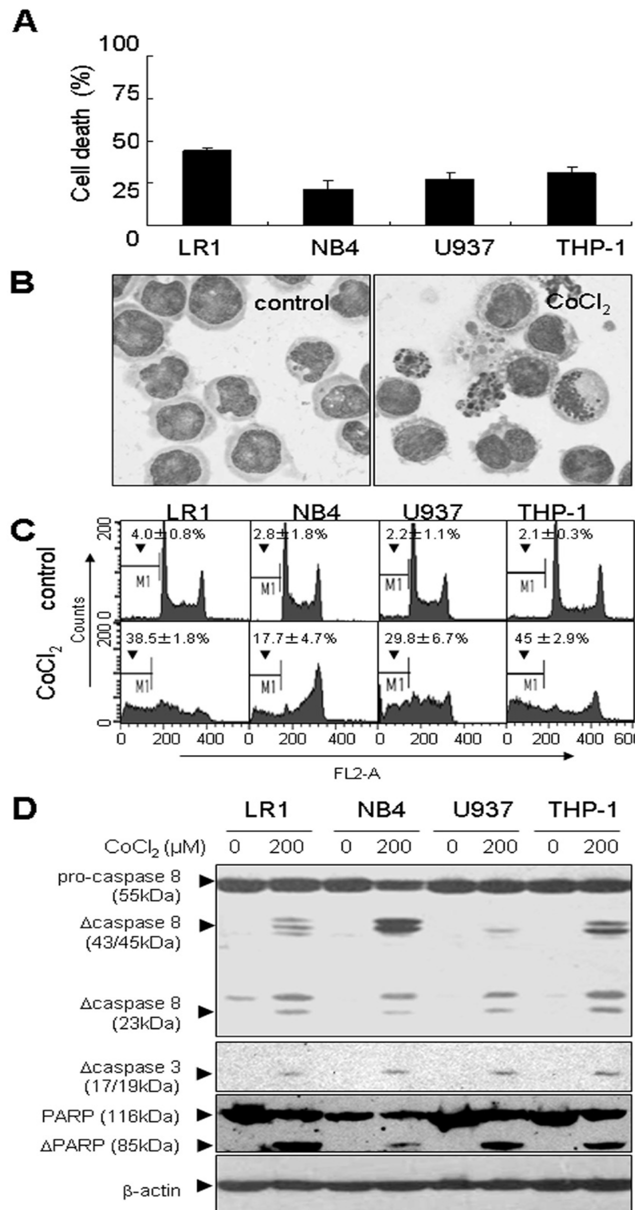

Fig. (1). $CoCl_2$ induces apoptosis in leukemic cells. NB4, NB4-LR1 (LR1), U937, and THP-1 cells were treated with 200 μM of $CoCl_2$ for 24 hours. (A) Cell death was evaluated by Trypan blue exclusion assay. Data are means ± SD (bars) values from three separate experiments. (B) A representative morphological changes observed under microscope (magnificent, ×1000) for THP-1 cells without and with $CoCl_2$ treatment. (C) The representative histograms showing distributions of DNA content were presented and the percentages of sub-G_1 cells were given with means ± S.D of triplicates in an independent experiment. (D) Western blots analysis of caspase 8, cleaved caspase 3, and PARP. Activation of caspase 8 is characterized by the appearance of bands at 43/45 and 23 kDa. PARP and the appearance of a fragment of 85 kDa proteolytic fragment were clearly shown in $CoCl_2$ treated cells. Blots were stripped and reprobed with β-actin to verify equal loading. Δ represents the cleaved fragments. All experiments were repeated for three times and the same results were obtained.

to modify c-myc expression, because c-myc affects hTERT expression via transactivation of hTERT gene [14, 21, 22]. The results demonstrated that $CoCl_2$ also resulted in a substantial decrease in c-myc protein in all the cells tested (Fig. **2A**), suggesting that downregulation of hTERT is likely to be linked to the decrease of c-myc.

OVEREXPRESSION OF HTERT PROTECTS NB4-LR1 CELLS FROM $COCL_2$ INDUCED APOPTOSIS

As documented [23, 24], telomerase expression prevents cells from apoptosis and plays a role in cellular resistance independently of its activity on telomeres. Given that $CoCl_2$ could downregulate hTERT expression and that telomerase is endowed with an anti-apoptotic function, we asked whether ectopic expression of hTERT might protect cells from $CoCl_2$-induced apoptosis. Based on this hypothesis, further investigation was performed on the retroviral transduced NB4-LR1/hTERT-GFP cells overexpressing both hTERT and GFP reporter from the same transcript and the NB4-LR1/GFP cells infected with the control virus and expressing only the GFP protein. Because these two engineering sublines were more sensitive to $CoCl_2$-induced apoptosis than their parental NB4-LR1 cells (data not shown), 100 μM $CoCl_2$ was applied to treat both cell lines for 2 days. As in NB4-LR1 cells, $CoCl_2$ treatment induced HIF-1α accumula-

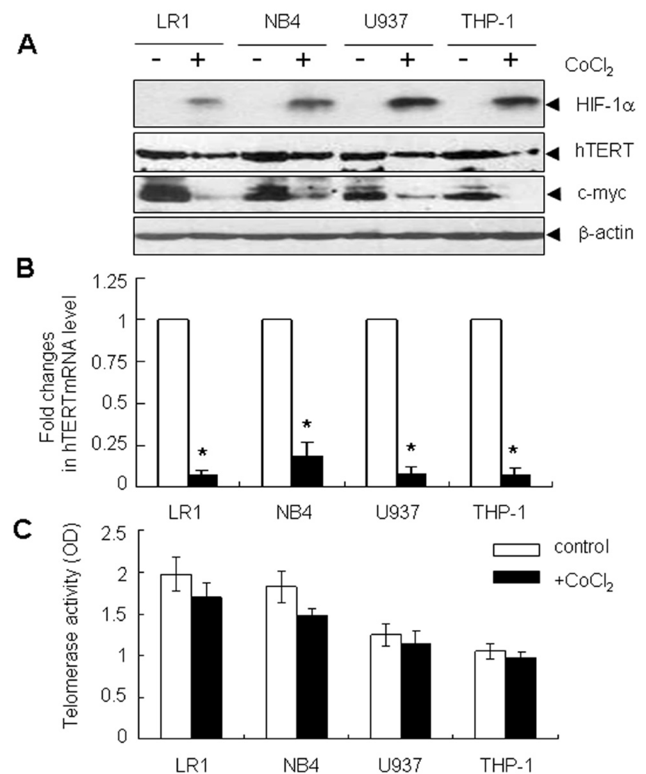

Fig. (2). $CoCl_2$ downregulates expression of hTERT. The indicated cells were treated with 200μM of $CoCl_2$ for 24 hours. (A) hTERT, c-myc, and HIF-1α proteins were detected by Western blots with β-actin as equal loading control. (B) hTERT mRNA expression was quantified using real-time quantitative PCR. The hTERT mRNA abundance in each control was set as 1. The values represent means of relative levels of the overall hTERT mRNA with bars as SD of triplicate samples. Symbol * represents P<0.01 compared with the corresponding $CoCl_2$-untreated cells. (C) Telomerase activity was assessed using a TeloTAGGG Telomerase PCR ELISA kit and arbitrarily expressed as absorbance (A_{450}-A_{630}). All experiments were repeated three times.

tion associated with both hTERT and c-myc downregulation in NB4-LR1/GFP cells. We found that the ectopic hTERT expression significantly protected NB4-LR1/hTERT-GFP cells from $CoCl_2$ induced toxicity, as evidenced by cell viability (Fig. **3A**), morphology (data not shown), DNA content (sub-G_1) (Fig. **3B**), caspase activation, and PARP cleavage (Fig. **3C**). Of note, hTERT ectopic expression change neither HIF-1α accumulation nor c-myc downregulation induced by $CoCl_2$ (Fig. **3D**).

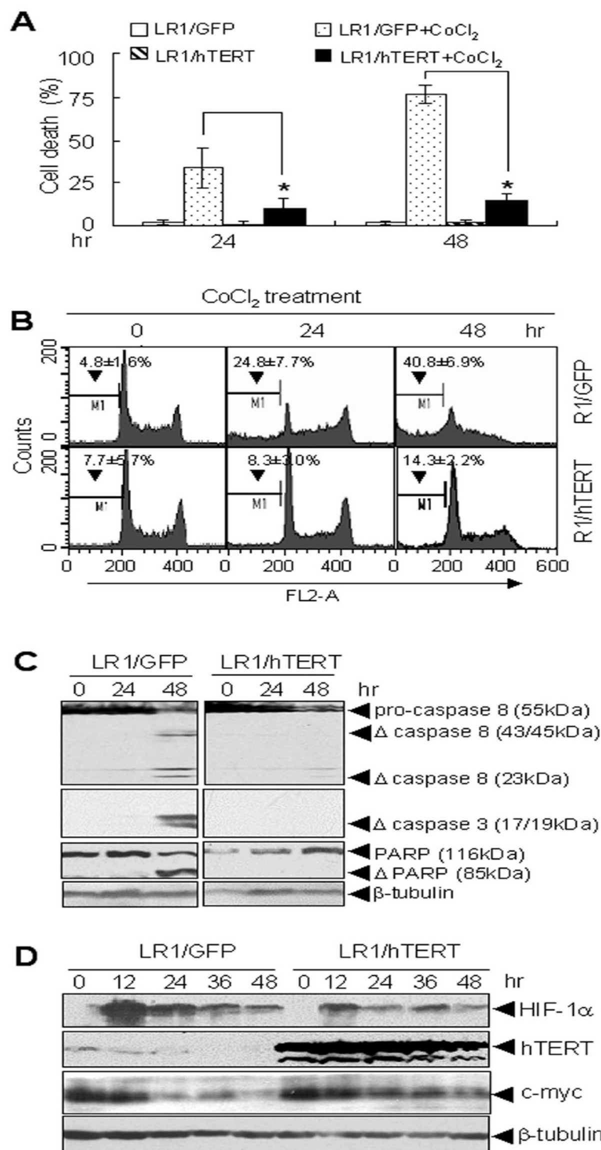

Fig. (3). Ectopic expression of hTERT blocks $CoCl_2$-induced apoptosis. NB4-LR1/GFP (LR1/GFP) and NB4-LR1/hTERT-GFP (LR1/hTERT) cells were treated with 100μM $CoCl_2$ for 2 days. (A) Cell death was evaluated by Trypan blue exclusion assay. Data are means ± SD (bars) values from three separate experiments. Symbol * represents P<0.01 compared between $CoCl_2$ treated LR1/GFP and LR1/hTERT cells. (B) The representative histograms showing distribution of DNA content and the percentages of sub-G_1 cells were given with means ± SD of triplicates in an independent experiment. (C) Western blots analysis of caspase 8, cleaved caspase 3 and PARP with β-tubulin as a loading control, Δ represents the cleaved fragments. (D) hTERT, HIF-1α and c-myc proteins were detected by Western blots with β-tubulin as loading control.

INDUCIBLE HIF-1α OVEREXPRESSION REPRESS HTERT WHEREAS DEPLETION OF HIF-1α FAILS TO AFFACT $COCL_2$-INDUCED HTERT DOWNREGULATION

In order to determine the role of HIF-1α in $CoCl_2$-induced downregulation of hTERT, a HIF-1α inducible ($U937T^{pool}$) and the vector transfected control cell line ($U937T^{empty}$) [18] were used as described previously [18], the expression of HIF-1α was significantly induced after tetracycline withdrawal in $U937T^{pool}$ but not in $U937T^{empty}$ subline (Fig. **4A**). More intriguingly, hTERT transcripts were downregulated with the HIF-1α induction (Fig. **4B**) and a slight decrease of hTERT protein could be seen (Fig. **4A**). However this downregulation is delayed suggesting that it is probably not the result of a direct action of HIF-1α on hTERT promoter. By the way, the expression of c-myc protein decreased concomitantly with HIF-1α induction.

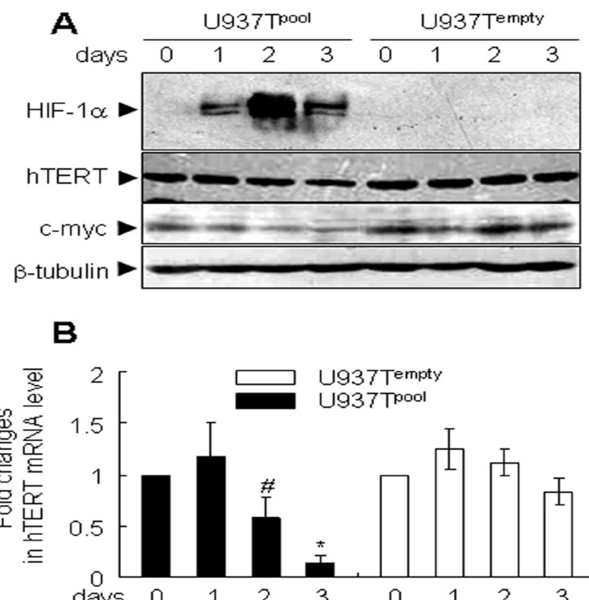

Fig. (4). HIF-1α inducible overexpression represses hTERT expression. (A) $U937T^{pool}$ and $U937T^{empty}$ were grown for the indicated days after withdrawal of tetracycline. HIF-1α, hTERT, and c-myc proteins were detected by Western blots with β-tubulin as a loading control. (B) hTERT mRNA in $U937T^{pool}$ and $U937T^{empty}$ cells was quantified by real-time PCR. Columns: relative levels of the overall hTERT mRNA; bars: SD. * and # represent P<0.01 and P<0.05 compared to control cells, respectively.

Next, we inhibited HIF-1α expression using shRNA specifically targeting HIF-1α mRNA in U937 cells (U937-shR-α14). As shown in Fig. **5A**, knocking down HIF-1α inhibited accumulation of HIF-1α after treatment of U937-shR-α14 cells with 200μM $CoCl_2$ compared with the U937-shR-NC cells. However, silencing of HIF-1α did not block $CoCl_2$-induced downregulation of hTERT at both mRNA (Fig. **5B**) and protein levels (Fig. **5A**). Furthermore, $CoCl_2$ treatment decreased c-myc protein level in both U937-shR-α14 and U937-shR-NC cells independently of the HIF-1α expression. Of note trypan blue exclusion assay and flow cytometric analysis showed that $CoCl_2$-induced toxicity and apoptosis were similar in both cell lines (Fig. **5C** and **5D**). Taken together, these results suggested that HIF-1α is not

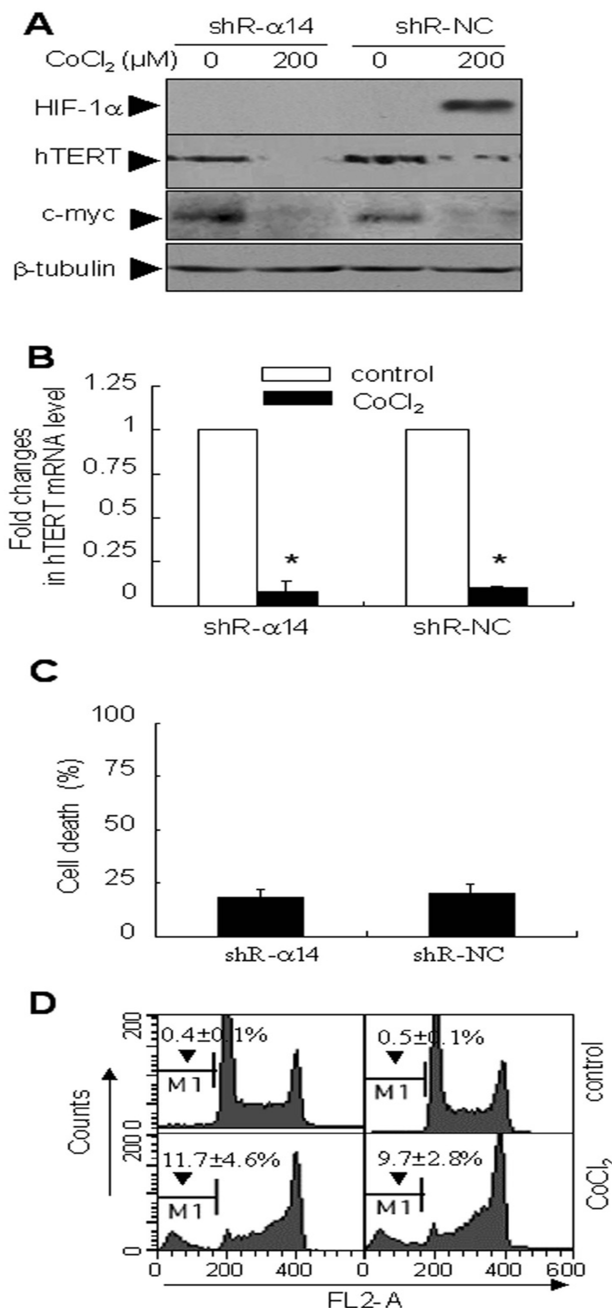

Fig. (5). The silence of HIF-1α by shRNA did not abrogate $CoCl_2$-induced downregulation of hTERT expression. U937-shR-α14 cells expressing a shRNA specifically directed against HIF-1α and the vector-transfected control cells (U937-shR-NC) were treated with 200 μM $CoCl_2$ for 24 hours. (A) HIF-1α, hTERT, and c-myc proteins were detected by Western blots. (B) hTERT mRNA was measured using real-time quantitative PCR. Columns: relative levels of the overall hTERT mRNA; bars: SD. Symbol * represents $P<0.01$ compared with the corresponding $CoCl_2$-untreated cells. (C) Cell death was evaluated by Trypan blue exclusion assay. Data are means ± SD (bars) values from three separate experiments. (D) The representative histograms showing distributions of DNA content were presented and the percentages of sub-G_1 cells were given with means ± SD of triplicates in an independent experiment.

indispensable for $CoCl_2$-induced hTERT decrease and c-myc most likely mediated this downregulation.

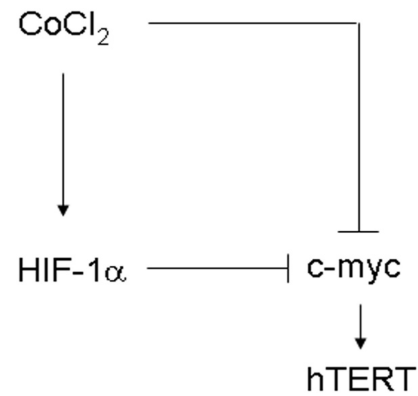

Fig. (6). A speculative sketch of mechanisms of $CoCl_2$–induced downregulation of hTERT in leukemic cells. Hypoxia-mimetic agent $CoCl_2$ downregulates the expression of c-myc in a HIF-1α dependent and independent pathways, which in turn downregulates the expression of hTERT.

DISCUSSION

Emerging evidence suggests that telomerase has additional extra telomeric roles in mediating cell survival and anti-apoptotic functions against various cytotoxic stresses [17, 25, 26]. As reported, expression of dominant negative hTERT (DN-hTERT) or knockdown of hTERT by siRNA or differentiation inducing agents such as ATRA, could induce apoptosis, loss of tumorigenicity, and enhance effects of anticancer drugs in various cancer cells [3, 23, 24]. On the contrary, overexpression of wild type hTERT (WT-hTERT) could increase resistance to DNA damage-related signals [27].

Others and we have reported that $CoCl_2$, a commonly used hypoxia-mimetic agent, could induce apoptosis in different cell lines including glioma, cervical and leukemic cancer cells [13-15]. Both the mitochondrial and cell death receptor mediated pathway are involved in $CoCl_2$ induced apoptosis. Given the possible role of hTERT in apoptosis, we investigate the effect of $CoCl_2$ on hTERT expression. We found that apoptosis inducing concentrations of $CoCl_2$ downregulated hTERT expression at both mRNA and protein levels. We therefore speculated that hTERT downregulation might contribute to $CoCl_2$ induced apoptosis and employed a stable hTERT overexpressing NB4-LR1 leukemic cell line. Indeed, overexpression of hTERT significantly inhibited $CoCl_2$ induced apoptosis in NB4-LR1 cells. These data suggested that hTERT plays a role in $CoCl_2$ induced apoptosis.

The relationship between telomerase activity in terms of telomere length maintenance and anti-apoptotic activity of hTERT is controversial. Some reports showed hTERT lacking its reverse transcriptase activity also loses its cytoprotective activity while others reported the anti-apoptotic activity of hTERT is regardless of telomere elongation activity [17, 25, 26]. In agreement with the latter, no much significant decrease of telomerase activity were observed during $CoCl_2$ induced apoptosis.

$CoCl_2$ is known to block the degradation and thus induce the accumulation of HIF-1α protein. Furthermore, HIF-1α has been implicated in the transcriptional regulation of the hTERT gene expression and telomerase activity. However, controversial results were obtained in different studies. Sev-

eral groups demonstrated that HIF-1α could directly bind to the promoter of hTERT and upregulate the expression of hTERT while introduction of an antisense oligonucleotide for HIF-1α diminishes hTERT expression during hypoxia, indicating that upregulation of hTERT by hypoxia is directly mediated by HIF-1α [8]. On the other hand, one group reported that HIF-1α may downregulate hTERT expression by functional counteracting c-myc, which is independent of its transcriptional activity or DNA binding activity [11]. In addition, Seimiya *et al.* found that hypoxia enhanced telomerase activity in the solid tumor A2780 and HT-29 cells but not in the leukemic U937 cells [7]. These studies reveal a complex relationship between HIF-1α and hTERT expression, suggesting that function of HIF-1α in leukemic cell lines is different from that in solid tumor and the effect of HIF-1α on hTERT regulation may depend on the cell context. Furthermore, it has been recently shown that HIF-2α, a paralog of HIF-1α either enhances or represses hTERT transcription depending on the cell types [28].

To explore the role of HIF-1α in $CoCl_2$-induced hTERT downregulation during apoptosis in leukemic cells, we established both HIF-1α inducible expression and HIF-1α knockdown cell lines. Induction of HIF-1α expression decreased the expression of hTERT without any consequences on cell viability. In contrast and unexpectedly, HIF-1α knockdown didn't abrogate $CoCl_2$-induced hTERT downregulation during apoptosis. These results demonstrate that HIF-1α is not indispensable for $CoCl_2$-induced downregulation of hTERT in leukemic cell lines.

There are two c-myc, five Sp1, one Ets, and two Inr binding sites present at the hTERT core promoter [16, 29]. Out of all these transcription factors, c-myc was the most extensively studied positive regulator of hTERT transcription in various cell lines [8, 30-32]. We found that c-myc was reduced by $CoCl_2$ in all the treated cells and induction of HIF-1α reduced the expression of c-myc. However, silence of HIF-1α expression could not rescue $CoCl_2$-induced decrease of c-myc suggesting other pathways are involved in this process. Given the key role of c-myc in the regulation of hTERT, we speculate that the decreasing of c-myc might responsible for $CoCl_2$ induced down-regulation of hTERT.

CONCLUSION

In summary, the present study provides evidence that downregulation of hTERT contributes to $CoCl_2$ induced apoptosis in leukemia cell lines. C-myc is most likely involved in this process and $CoCl_2$ could trigger its downregulation either by HIF-1α dependent and independent way, which warrant further investigation (Fig. **6**).

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REFERENCES

[1] Wai LK. Telomeres, telomerase, and tumorigenesis--a review. MedGenMed 2004; 6: 19.

[2] Fridlender ZG, Cohen PY, Golan O, Arish N, Wallach-Dayan S, Breuer R. Telomerase activity in bleomycin-induced epithelial cell apoptosis and lung fibrosis. Eur Respir J 2007; 30: 205-13.

[3] Tarkanyi I, Dudognon C, Hillion J, *et al*. Retinoid/arsenic combination therapy of promyelocytic leukemia: induction of telomerase-dependent cell death. Leukemia 2005; 19: 1806-11.

[4] Zhang Y, Cao EH, Liang XQ, Qin JF. Increasing sensitivity to arsenic trioxide-induced apoptosis by altered telomere state. Eur J Pharmacol 2003; 474: 141-7.

[5] Melillo G, Semenza GL. Meeting report: exploiting the tumor microenvironment for therapeutics. Cancer Res 2006; 66: 4558-60.

[6] Gordan JD, Simon MC. Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr Opin Genet Dev 2007; 17: 71-7.

[7] Seimiya H, Tanji M, Oh-hara T, Tomida A, Naasani I, Tsuruo T. Hypoxia up-regulates telomerase activity via mitogen-activated protein kinase signaling in human solid tumor cells. Biochem Biophys Res Commun 1999; 260: 365-70.

[8] Nishi H, Nakada T, Kyo S, Inoue M, Shay JW, Isaka K. Hypoxia-inducible factor 1 mediates upregulation of telomerase (hTERT). Mol Cell Biol 2004; 24: 6076-83.

[9] Minamino T, Mitsialis SA, Kourembanas S. Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. Mol Cell Biol 2001; 21: 3336-42.

[10] Anderson CJ, Hoare SF, Ashcroft M, Bilsland AE, Keith WN. Hypoxic regulation of telomerase gene expression by transcriptional and post-transcriptional mechanisms. Oncogene 2006; 25: 61-9.

[11] Koshiji M, Kageyama Y, Pete EA, Horikawa I, Barrett JC, Huang LE. HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. Embo J 2004; 23: 1949-56.

[12] Huang Y, Du KM, Xue ZH, *et al*. Cobalt chloride and low oxygen tension trigger differentiation of acute myeloid leukemic cells: possible mediation of hypoxia-inducible factor-1alpha. Leukemia 2003; 17: 2065-73.

[13] Guo M, Song LP, Jiang Y, Liu W, Yu Y, Chen GQ. Hypoxia-mimetic agents desferrioxamine and cobalt chloride induce leukemic cell apoptosis through different hypoxia-inducible factor-1alpha independent mechanisms. Apoptosis 2006; 11: 67-77.

[14] Zou W, Yan M, Xu W, *et al*. Cobalt chloride induces PC12 cells apoptosis through reactive oxygen species and accompanied by AP-1 activation. J Neurosci Res 2001; 64: 646-53.

[15] Jung JY, Kim WJ. Involvement of mitochondrial- and Fas-mediated dual mechanism in CoCl2-induced apoptosis of rat PC12 cells. Neurosci Lett 2004; 371: 85-90.

[16] Zou W, Zeng J, Zhuo M, *et al*. Involvement of caspase-3 and p38 mitogen-activated protein kinase in cobalt chloride-induced apoptosis in PC12 cells. J Neurosci Res 2002; 67: 837-43.

[17] Dudognon C, Pendino F, Hillion J, Saumet A, Lanotte M, Segal-Bendirdjian E. Death receptor signaling regulatory function for telomerase: hTERT abolishes TRAIL-induced apoptosis, independently of telomere maintenance. Oncogene 2004; 23: 7469-74.

[18] Song LP, Zhang J, Wu SF, *et al*. Hypoxia-inducible factor-1alpha-induced differentiation of myeloid leukemic cells is its transcriptional activity independent. Oncogene 2007.

[19] Yatabe N, Kyo S, Maida Y, *et al*. HIF-1-mediated activation of telomerase in cervical cancer cells. Oncogene 2004; 23: 3708-15.

[20] Xu D, Gruber A, Peterson C, Pisa P. Supression of telomerase activity in HL60 cells after treatment with differentiating agents. Leukemia 1996; 10: 1354-7.

[21] Greenberg RA, O'Hagan RC, Deng H, *et al*. Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. Oncogene 1999; 18: 1219-26.

[22] Wang J, Xie LY, Allan S, Beach D, Hannon GJ. Myc activates telomerase. Genes Dev 1998; 12: 1769-74.

[23] Xi L, Chen G, Zhou J, *et al*. Inhibition of telomerase enhances apoptosis induced by sodium butyrate via mitochondrial pathway. Apoptosis 2006; 11: 789-98.

[24] Massard C, Zermati Y, Pauleau AL, *et al*. hTERT: a novel endogenous inhibitor of the mitochondrial cell death pathway. Oncogene 2006; 25: 4505-14.

[25] Del Bufalo D, Rizzo A, Trisciuoglio D, *et al*. Involvement of hTERT in apoptosis induced by interference with Bcl-2 expression and function. Cell Death Differ 2005; 12: 1429-38.

[26] Fu W, Begley JG, Killen MW, Mattson MP. Anti-apoptotic role of telomerase in pheochromocytoma cells. J Biol Chem 1999; 274: 7264-71.

[27] Zhang P, Chan SL, Fu W, Mendoza M, Mattson MP. TERT suppresses apoptotis at a premitochondrial step by a mechanism requiring reverse transcriptase activity and 14-3-3 protein-binding ability. FASEB J 2003; 17: 767-9.

[28] Lou F, Chen X, Jalink M, *et al*. The opposing effect of hypoxia-inducible factor-2alpha on expression of telomerase reverse transcriptase. Mol Cancer Res 2007; 5: 793-800.

[29] Cong YS, Wen J, Bacchetti S. The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. Hum Mol Genet 1999; 8: 137-42.

[30] Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 2005; 6: 635-45.

[31] Wu KJ, Grandori C, Amacker M, *et al*. Direct activation of TERT transcription by c-MYC. Nat Genet 1999; 21: 220-4.

[32] Greenberg RA, O'Hagan RC, Deng H, *et al*. Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. Oncogene 1999; 18: 1219-26.

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