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Arsenic trioxide suppresses transcription of hTERT through down-regulation of multiple transcription factors in HL-60 leukemia cells

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HIGHLIGHTS

- Arsenic trioxide (ATO) decreased the expression of hTERT at mRNA and protein levels.
- Arsenic trioxide suppressed the expression of Sp1, c-Myc, NF-κB and USF2.
- Inhibiting Sp1, c-Myc, NF-κB and USF2 potentiates antitumor activity of ATO.

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ABSTRACT

Acute promyelocytic leukemia (APL) is largely caused by the *t*(15,17) chromosome translocation, leading to the production of the PML/retinoic acid receptor alpha fusion. All-trans retinoic acid (ATRA) and arsenic trioxide (ATO), as a monotherapy or combination therapy, have been successfully used to treat APL primarily by targeting the degradation of the fusion protein. We previously observed that ATO treatment induced cell death in APL cell line HL-60 accompanied by inhibition of the human telomere reverse transcriptase (hTERT) activity, a critical enzyme responsible for the control of cell replication and transformation in cancer cells. In the present study, we investigated the underlying mechanism by which hTERT activity is inhibited by ATO in HL-60 cells. Our results showed that ATO down-regulated the expression of hTERT at both mRNA and protein levels. Further molecular analysis revealed that the expression of four transcription factors Sp1, c-Myc, NF-κB and USF2, which are located in the proximate promoter region (−1126 to −47) of hTERT, was also suppressed by ATO. Notably, we observed that down-regulation of these four factors by their siRNAs potentiates ATO-induced cell growth inhibition and apoptosis. Therefore, our results provide a novel mechanism of action of ATO for the treatment of APL.

1. Introduction

Acute promyelocytic leukemia (APL) is largely caused by the *t* (15,17) chromosomal translocation, leading to the production of the promyelocytic leukemia protein (PML)/retinoic acid receptor α (RARA) fusion (de The et al., 2012). Clinically, APL has been well managed with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) (Grimwade and Enver, 2004; Lengfelder et al., 2013a; Liu et al., 2012; Sanz et al., 2013). The efficacy of ATRA can be explained by its targets of the molecular lesion in APL whereas the mechanism of action of ATO in treating APL appears to be multifaceted. ATO is a poisonous agent, and the mechanism of arsenic cytotoxicity in APL may be involved in induced degradation of the PML–RARA fusion, targeting of PML to nuclear bodies, production of reactive oxygen species (ROS), collapse of the mitochondrial transmembrane potential, or cytogenetic changes

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(Chou and Dang, 2005; Liu et al., 2012; Yaghmaie et al., 2012). We previously observed that treatment of HL-60 cells with ATO inhibited the telomerase activity (Zhang et al., 2003, 2008). Interestingly, several studies have suggested that arsenic cytotoxicity in APL cells may also be mediated by down-regulation of the human telomerase reverse transcriptase (hTERT) expression (Chou et al., 2005, 2001; Ghaffari et al., 2012; Li et al., 2009; Li and Liu, 2003; Tong et al., 2005), though the underlying mechanism remains to be investigated.

Telomeres are tandem sequences with hundreds to thousands copies at the ends of eukaryotic chromosomes. Telomeres play a crucial role in maintaining genome stability and in controlling the replication potential in somatic cells (Ding et al., 2013). In normal human somatic cells, telomeres shorten with each cell division, contributing to limited cell replications. However, many cancer cells develop a mechanism to escape from this limitation by overexpressing telomerase, an enzyme that maintains the telomere length (Ding et al., 2013; Holysz et al., 2013; Xu et al., 2013). Telomerase constitutes three major proteins, and hTERT is the major determinant of the telomerase activity. In many tumor cells, the hTERT is abnormally activated or overexpressed whereas it is inactivated in most normal human cells (Xu et al., 2013). Although regulation of hTERT expression occurs at multiple levels, transcriptional regulation of hTERT expression appears to be the major mechanism (Gladych et al., 2011). Consistent with this notion, several recent reports also suggest that mutations in the promoter region of hTERT contribute to hTERT overexpression in multiple cancers (Horn et al., 2013; Huang et al., 2013; Killela et al., 2013; Tallet et al., 2014). These observations indicate the importance of hTERT overexpression in the development and maintenance of cancers, and suggest that targeting hTERT can be developed as an anti-cancer therapy (Lu et al., 2012). In fact, many cancer therapeutics can exhibit their effect by suppressing the expression of hTERT (Lu et al., 2012). Here we show that ATO suppresses the transcription of hTERT through down-regulation of multiple transcription factors in HL-60 cells.

2. Materials and methods

2.1. Cell culture

HL-60 cells were cultured in RPMI-1640 containing 10% newborn bovine serum supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37 °C and 5% CO_2. Cells were split every 2–3 days to avoid overgrowth.

2.2. Western blotting

To determine the protein level of hTERT and transcription factors Sp1, Nuclear factor-κB (NF-κB), Upstream stimulatory factor (USF) and c-Myc, HL-60 cells cultured in 10 cm dishes were treated with various concentrations of ATO (Sigma, St. Louis, Missouri) for 48 h, and harvested for western blotting. Total cell lysate was prepared in the lysis buffer (50 mM Tris–Cl, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, and protease inhibitors). Approximately 50 μg of total lysate was used to run 10% SDS-PAGE and immunoblotting was performed with antibodies against hTERT (Abcam), Specificity protein 1 (Sp1, Cell Signaling Technology, Beverly, MA), NF-κB (p65, Ando Biotechnology, San Francisco, CA), USF2 (American Affinity), and c-Myc (Ando Biotechnology, San Francisco, CA) with the enhanced chemiluminescence (ECL) kit (Millipore) as described before (Wu et al., 2013).

2.3. Luciferase reporter gene assays

To determine whether ATO has any direct impact on the hTERT promoter activity and to map the responsive region in the promoter, four different luciferase reporter genes were constructed. Briefly, genomic DNA was isolated using the E.Z.N.A.® Tissue DNA Kit (OMEGA Bio-Tek, San Diego, California) from HL-60 cells, and PCR amplification was employed to amplify the promoter regions that span −1126, −793, −461, or −281 to −47, and the PCR products were subcloned into pMD18-T. The inserts were then released by *Kpn* I and *Hind* III from pMD18-T and subcloned into the pGL3 luciferase reporter gene vector (Promega, Fitchburg, Wisconsin). The resulting reporter gene constructs were verified by DNA sequencing. To perform luciferase reporter gene assays, 0.5 μg of each luciferase reporter gene construct or pGL3 vector control only along with 0.05 μg of internal reference plasmid pRL-TK were cotransfected into HL-60 cells using Lipofectamine™ LTX Reagent (Invitrogen, Grand Island, New York) according to manufacture's instruction. Twenty-four hours after transfection, cells were treated with various concentrations of ATO or vehicle control for 48 h and harvested for luciferase activity measurement using Dual-Luciferase Assay Kit (Promega, Fitchburg, Wisconsin). The relative luciferase activity of Firefly luciferase over Renilla luciferase was normalized to the vector control for statistical analysis from three independent experiments.

2.4. Real-time PCR and RT-PCR

To determine the effect of ATO on the expression of hTERT mRNA and several transcription factors, real-time quantitative PCR (qPCR) or reverse transcription PCR (RT-PCR) was performed. Briefly, total RNA was extracted from HL-60 cells treated with various concentrations of ATO for 48 h using Trizol Reagent (Invitrogen, Grand Island, New York). RT-PCR or qPCR was performed by following the protocols reported previously using qPCR machine (ABI 7500), and qPCR results were quantitated using the $2^{-\Delta\Delta CT}$ algorithm (Livak and Schmittgen, 2001). The primers used for qPCR or RT-PCR were as follow: Sp1 forward, 5′-GGT TTA CAA AGG AGG CTA CAG A-3′; Sp1 reverse, 5′-CCT CAC CCC CCA CTC TTA G-3; c-Myc forward, 5′-TCG GAT TCT CTG CTC TCC TC-3′; c-Myc reverse, 5′-TCG GTT GTT GCT GAT CTG TC-3′; NF-κB (p65) forward, 5′-AGG ACA TAT GAG ACC TTC AAG AGC-3′; NF-κB reverse, 5′-CTC ATC ATA GTT GAT GGT GCT CAG-3′; USF2 forward, 5′-AGG GAC CAG AAA CAA GAG G-3′; USF2 reverse, 5′-TAG TCC TCT CAC CTG GAG GC-3′; Myeloid zinc finger1(MZF1) forward, 5′-CCG GAG ATG GGT CAC AGT CC-3′; MZF1 reverse, 5′-CAG TAA ACG AGC AGG TAC TC-3′; GATA1 forward, 5′-CAG TAA ACG AGC AGG TAC TC-3′; GATA1 reverse 5′-CAT AAA GCC ACC AGC TGG TC-3′; Myoblast determination protein 1 (MYOD1) forward, 5′-ATG ACG ACC CGT GTT T-3′; MYOD1 reverse, 5′-CCG CTG GTT TGG ATT-3′; AP-2 alpha forward, 5′-ACC AGC AAC GGG ACG GCA CGG-3′; AP-2 alpha reverse, 5′-TGG CGG AGA CAG CAT TGC TGT TG-3′; β-actin forward, 5′-GCT CAC CAT GGA TGA TGA TAT C-3′; β-actin reverse, 5′-GCC AGA TTT TCT CCA TGT CGT C-3′; GADPH forward, 5′-CAA GGT CAT CCA TGA CAA CTT TG-3′; GADPH reverse, 5′-GTC CAC CAC CCT GTT GCT GTA G-3′.

2.5. Transfection

Cells were transfected with specific siRNA (NF-κB siRNA, SP1 siRNA, C-Myc siRNA, or USF2 siRNA), or control siRNA (Genepharma, Shanghai, China) using Lipofectamine 2000 for 48 h as described before (Yang et al., 2014a).