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Induction of apoptosis and inhibition of telomerase activity by trichostatin A, a histone deacetylase inhibitor, in human leukemic U937 cells

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Abstract

The objective of the present study was to investigate the effect of trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, on the cell growth and apoptosis and its effect on the telomerase activity in human leukemic cell line U937. Exposure of U937 cells to TSA resulted in growth inhibition and induction of apoptosis in a dose-dependent manner as measured by hemocytometer counts, fluorescence microscopy, agarose gel electrophoresis and flow cytometry analysis. The increase in apoptosis was associated with the up-regulation in proapoptotic Bax expression and down-regulation of antiapoptotic Bcl-2 and Bcl-X_L. TSA treatment inhibited the levels of cIAP family members and induced the proteolytic activation of caspase-3, which was associated with concomitant degradation of poly(ADP-ribose)-polymerase and β-catenin protein. TSA treatment markedly inhibited the activity of telomerase in a dose-dependent fashion. Additionally, the expression of human telomerase reverse transcriptase (hTERT), a main determinant of the telomerase enzymatic activity, was progressively down-regulated by TSA treatment. We therefore conclude that TSA demonstrated antiproliferative and apoptosis-inducing effects on U937 cells in vitro, and that changes in Bcl-2 family protein levels as well as telomerase activity may play an important role in its mechanism of action.

Keywords: TSA; Apoptosis; Bcl-2; IAPs; Caspase; Telomerase

Introduction

Modulation of chromatin structure through histone acetylation/deacetylation is known to be one of the major mechanisms involved in the regulation of gene expression. Two opposing enzyme activities determine the acetylation state of histones: histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively acetylating or deacetylating the epsilon-amino groups of lysine residues located in the amino-terminal tails of the histones. In general, transcriptionally active chromatin is associated with hyperacetylated histones, while silenced chromatin is linked to hypoacetylated histones (Monneret, 2005; Peterson and Laniel, 2004). Thus, inhibition of HDACs represents a new strategy in human cancer therapy since these enzymes play a fundamental role in regulating gene expression and chromatin assembly (Mei et al., 2004; Arts et al., 2003).

Eukaryotic chromosomes terminate in specialized nucleic acid–protein complexes known as telomeres. Disruption of telomere structure, by erosion of telomeric DNA or loss of telomere binding protein function, activates a signal transduction program that closely resembles the cellular responses generated upon DNA. Telomere dysfunction in turn induces a permanent proliferation arrest known as senescence and apoptotic cell death damage (Oulton and Harrington, 2000; Hahn and Meyerson, 2001). Most tumor cells have mechanisms that compensate for telomere shortening, most commonly through

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Table 1
Oligonucleotides used in RT-PCR

Name		Sequence of primers
Bax	Sense	5′-ATG-GAC-GGG-TCC-GGG-GAG-3′
	Antisense	5′-TGG-AAG-AAG-ATG-GGC-TGA-3′
Bcl-2	Sense	5′-CAG-CTG-CAC-CTG-ACG-3′
	Antisense	5′-GCT-GGG-TAG-GTG-CAT-3′
Bcl-X_L	Sense	5′-CAG CTG CAC CTG ACG-3′
	Antisense	5′-GCT GGG TAG GTG CAT-3′
XIAP	Sense	5′GAA-GAC-CCT-TGG-GAA-CAA-CA-3′
	Antisense	5′-CGC-CTT-AGC-TGC-TCT-CTT-CAG-T-3′
cIAP-1	Sense	5′-TGA-GCA-TGC-AGA-CAC-ATG-C-3′
	Antisense	5′TGA-CGG-ATG-AAC-TCC-TGT-CC-3′
cIAP-2	Sense	5′-CAG-AAT-TGG-CAA-GAG-CTG-G-3′
	Antisense	5′-CAC-TTG-CAA-GCT-GCT-CAG-G-3′
Fas	Sense	5′-TCT-AAC-TTG-GGG-TGG-CTT-TGT-CTT-C-3′
	Antisense	5′-GTG-TCA-TAC-GCT-TTC-TTT-CCA-T-3′
FasL	Sense	5′-GGA-TTG-GGC-CTG-GGG-ATG-TTT-CA-3′
	Antisense	5′AGC-CCA-GTT-TCA-TTG-ATC-ACA-AGG-3′
hTERT [a]	Sense	5′-AGC-CAG-TCT-CAC-CTT-CAA-CC-3′
	Antisense	5′-GTT-CTT-CCA-AAC-TTG-CTG-ATG-3′
TEP-1 [b]	Sense	5′-TCA-AGC-CAA-ACC-TGA-ATC-TGA-G-3′
	Antisense	5′-CCC-CGA-GTG-AAT-CTT-TCT-ACG-C-3′
hTR [c]	Sense	5′-TCT-AAC-CCT-AAC-TGA-GAA-GGG-CGT-AG-3′
	Antisense	5′-GTT-TGC-TCT-AGA-ATG-AAC-GGT-GGA-AG-3′
GAPDH [d]	Sense	5′-CGG-AGT-CAA-CGG-ATT-TGG-TCG-TAT-3′
	Antisense	5′-AGC-CTT-CTC-CAT-GGT-GGT-GAA-GAC-3′

[a] Telomerase reverse transcriptase.
[b] Telomerase-associated protein-1.
[c] Telomeric repeat binding factor.
[d] Glyceraldehyde-3-phosphate dehydrogenase.

the activation of telomerase, allowing them to stably maintain their telomeres and grow indefinitely. These observations suggest that telomerase reactivation is a rate-limiting step in cellular immortality and carcinogenesis, and telomerase repression can act as a tumor-suppressive mechanism (Takakura et al., 1998; Kyo et al., 1999; Hahn and Meyerson, 2001). However, the molecular mechanisms by which telomerase activity is regulated in concordance with cell growth properties remain unclear.

This study was performed to elucidate further the mechanisms of the apoptotic pathway by HDAC inhibitor trichostatin A (TSA) and its effect on telomerase activity in a human leukemic cell line U937. We report here that exposure of U937 cells to TSA resulted in a dose-dependent growth inhibition and apoptosis. This increase in apoptosis by TSA was associated with an increase in Bax expression and an activation of caspase-3. Furthermore, down-regulation of hTERT expression by TSA treatment was associated with an inhibition of telomerase activity.

Materials and methods

Cell culture, TSA and cell proliferation assay

The human leukemia cell line U937 was purchased from the American Type Culture Collection (Rockville, MD) and maintained at 37°C in a humidified condition of 95% air and 5% CO_2 in DMEM (Gibco BRL, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. TSA was purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in dimethylsulfoxide. For growth inhibition analysis, cells were seeded and exposed to various concentrations of TSA for 48 h. The cells were trypsinized, washed with phosphate-buffered saline (PBS), and the viable cells were scored with a hemocytometer through exclusion of trypan blue.

Nuclear staining with DAPI

After treatment with TSA, the cells were washed with PBS and fixed with 3.7% paraformaldehyde (Sigma) in PBS for 10 min at room temperature. Fixed cells were washed with PBS and stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma) solution for 10 min at room temperature. The cells were washed two more times with PBS and analyzed via a fluorescence microscope.

Flow cytometric analysis

Cells were collected, washed with cold PBS and fixed in 75% ethanol at 4°C for 30 min. DNA contents of cells were measured using a DNA staining kit (CycleTEST™ PLUS Kit, Becton Dickinson, San Jose, CA). Propidium iodide (PI)-stained nuclear fractions were obtained by following the kit protocol. Fluorescence intensity was determined using a FACScan flow cytometer and analyzed by CellQuest software (Becton Dickinson).

RNA extraction and reverse transcriptase-PCR

Total RNA was prepared using an RNeasy kit (Qiagen, La Jolla, CA) and primed with random hexamers to synthesize complementary DNA using AMV reverse transcriptase (Amersham Corp., Arlington Heights, IL) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out in a Mastercycler (Eppendorf, Hamburg, Germany) with indicated primers in Table 1. Conditions for PCR reactions were 1 × (94°C for 3 min); 35 × (94°C for 45 s; 58°C for 45 s; and 72°C for 1 min) and 1 × (72°C for 10 min). Amplification products obtained by PCR were electrophoretically separated on 1% agarose gel and visualized by ethidium bromide (EtBr) staining.

Gel electrophoresis and Western blotting

The cells were harvested, lysed, and protein concentrations were quantified using the BioRad protein assay (BioRad Lab., Hercules, CA), following the

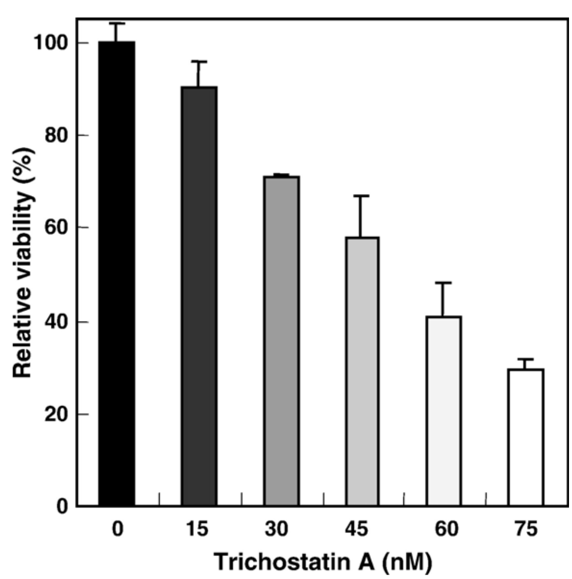

Fig. 1. Inhibition of the cell growth by TSA in U937 human leukemic cells. Cells were treated with variable concentrations of TSA for 48 h, and the viable cell number was determined by hemocytometer counts of trypan blue-excluding cells as described in Materials and methods. Results are expressed as the means ± SE of three independent experiments.