EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 31, No. 3, 126-133, September 1999

TGF-β1 inhibition of apoptosis through the transcriptional up-regulation of Bcl-X_L in human monocytic leukemia U937 cells

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Accepted 21 June 1999

Abbreviations: RT-PCR, reverse transcription-polymerase chain reaction; TβR, TGF-β1 receptor; SSCP, single strand conformational polymorphism; Cdk, cyclin-dependent kinase

Abstract

To characterize the TGF-β1 response of monocytic leukemia cells, we analyzed the effects of TGF-β1 on cell proliferation, differentiation, and apoptosis of human monoblastic U937 cells. Treatment of cells with TGF-β1 in the absence of growth factors significantly enhanced cell viability. Flow cytometric analysis of DNA content and CD14 expression revealed that TGF-β1 does not affect cell proliferation and differentiation. Consistent with these results was the finding that no transcriptional induction of Cdk inhibitors such as $p21^{Waf1}$, $p15^{Ink4b}$, and $p27^{Kip1}$ was detected following TGF-β1 treatment. Interestingly, however, pretreatment of TGF-β1 significantly inhibited Fas-, DNA damage-, and growth factor deprivation-induced apoptosis. This antiapoptotic effect was totally abrogated by anti-TGF-β1 antibody. Quantitative RT-PCR analysis demonstrated a dose- and time-dependent transcriptional up-regulation of Bcl-X_L, suggesting its implication in the TGF-1-mediated antiapoptotic pathway. We also observed elevated expression of c-Fos and PTEN/MMAC1. But, no detectable change was recognized in expression of c-Jun, Fas, Fadd, Fap-1, Bcl-2, and Bax. Taken together, our study shows that TGF-β1 enhancement of cellular viability is associated with its antiapoptotic effect, which may result from the transcriptional up-regulation of Bcl-X_L.

Keywords: TGF-β1, U937, apoptosis, Fas, Bcl-X_L, PTEN/MMAC1

Introduction

TGF-β1 is a multifunctional cytokine which plays a central role in regulating a broad spectrum of biological processes including cell proliferation, differentiation, and modulation of immune response (Massague, 1990). TGF-β1 exerts its various effects through serine/threonine kinase transmembrane receptors. TGF-β1 induced signaling from receptors to the nucleus is mediated by phosphorylation of cytoplasmic effector molecules, termed the Smad family of proteins (Graff *et al.*, 1996). Phosphorylated Smad2 and Smad3 form heteromeric complexes with Smad4 and then are translocated into the nucleus, where they function as transcriptional factors (Derynck and Zhang, 1996; Massague, 1996). Recent studies demon-strated that the TGF-β1-induced G_1 cell cycle arrest is partially attributed to the regulatory effects of TGF-β1 on the transcription and/or activities of G_1 cyclins, Cdks, and newly identified Cdk inhibitors such as $p15^{Ink4b}$, $p27^{Kip1}$, and $p21^{Waf1}$ (Hannon and Beach, 1994; Polyak *et al.*, 1994; Datto *et al.*, 1995).

TGF-β1 inhibits *in vitro* growth of a wide range of human epithelial and hemopoietic cells (Massague, 1990; Buske *et al.*, 1997). However, the resistance to TGF-β1-mediated growth inhibition, caused mainly by the mutational alteration of TGF-β1 receptors or Smad members, has been observed in a number of human malignancies including leukemias. This resistance to growth inhibition indicates that disruption of TGF-β1 signaling pathway may play an important role in tumor progression (Douglas *et al.*, 1997). TGF-β1 is a potent immunomodulatory molecule. TGF-β1 limits clonal expansion of activated lymphocytes by causing arrest in the G_1 phase of the cell cycle or inducing apoptotic cell death (Buske *et al.*, 1997). Recently, TGF-β1 was identified to function as a possible viability factor, involved in the generation of effector and/or long-lived memory T cells through the inhibition of activation-induced or Fas-mediated apoptosis (Cerwenka *et al.*, 1996). TGF-β1 inhibition of Fas-mediated apoptosis was also observed in murine bone marrow progenitor cells and human rheumatoid synovial cells (Kawakami *et al.*, 1996; Dybedal *et al.*, 1997). In monocytes, TGF-β1 was found to stimulate the production of inflammatory cytokines such as IL-6, IL-1β, and TNF-α early in inflam-

mation but subsequently to diminish the secondary monocyte response via down-regulation of the surface expression of CD32. TGF-β1-induced activities in monocytes suggest an important role of TGF-β1 in the fine-tuning of the immune response and in the regulation of immune complex-induced effector mechanism of monocytes (Reterink *et al.*, 1996). TGF-β1 also affects macrophagic differentiation and cellular adhesion of monocytes synergistically with vitamin D3 or TNF-α (Testa *et al.*, 1993; Lastres *et al.*, 1994).

Numerous studies have been performed on the regulatory role of TGF-β1 in cell proliferation and differentiation of leukemic cells (Taipale *et al.*, 1994; Micouin *et al.*, 1997; Defacque *et al.*, 1999). However, TGF-β1 effects on the apoptotic cell death and cellular viability of human monocytic leukemia cells are not well elucidated. In the present study, we investigated the TGF-β1 effects on cell proliferation, differentiation, and apoptosis of a human monoblastic myelogenous leukemia cell line, U937. Our studies showed that 1) TGF-β1 enhances the viability of the U937 cells through the inhibition of apoptotic cell death without affecting cell proliferation and differentiation; 2) TGF-β1 inhibition of apoptosis is accompanied by a significant transcriptional up-regulation of Bcl-X_L. These results suggest that the antiapoptotic effect of TGF-β1 might provide tumor cells a survival advantage and thus contribute to the malignant progression of human monoblastic leukemias.

Materials and Methods

Cell line and proliferation assay

A human monoblastic leukemia cell line U937 was obtained from American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS). Cells were seeded in 6-well tissue plates at an initial density of 1×10^4 cells/well in medium containing 10% FBS for 24 h. Various concentration of porcine recombinant TGF-β1 (R & D, Minneapolis, MN) was added in the presence of 1% FBS. Growth curves were obtained from cell counts with the hemocyto meter for 3 days at 24 h intervals. Three independent experiments were performed and the means were calculated.

Analysis of cell viability

5×10^5 cells were cultured in serum-deprived medium for 4 days in the presence or absence of TGF-β1 (2 ng/ml). Viable cells discriminated by trypan blue exclusion were counted at every 24 h using the hemocytometer. Three independent experiments were performed and the means were calculated.

Flow cytometric analysis of the cell cycle and apoptosis

5×10^5 cells were treated with TGF-β1 (2 ng/ml) for 72 h. The cells were harvested and fixed in 70% cold ethanol at 4°C for 1 h, and incubated in 1.5 ml PBS containing propidium iodide (100 μg/ml) and RNase (1 μg/ml) for 2 h at 37°C. For analysis of TGF-β1 effects on apoptotic cell death, cells were preincubated with various concentrations of TGF-β1 (0.1-10 ng/ml) for 0.5-48 h and then treated with 100 ng/ml of anti-Fas antibody (PharMingen, San Diego, CA) or 100 μM of etoposide (Sigma, St. Louis, MO) for 24 or 8 h, respectively. The DNA content of the cells and hypodiploid peak indicating apoptotic cells was analyzed using FACScan flow cytometry (Becton Dickinson, Mountain View, CA).

Analysis of CD14 expression

Expression of CD14, a marker for monocytic differentiation, was examined using FITC conjugated anti-CD14 antibody (Biosource, Camarillo, CA) and FITC conjugated goat anti-mouse IgG as control. TGF-β1 (2 ng/ml)-treated and control cells were washed twice with PBS, resuspended in 10 μg/ml of anti-CD14 antibody and incubated for 30 min on ice in the dark. The cells were analyzed using a FACScan flow cytometry (Becton Dickinson).

RNA extraction and cDNA synthesis

Total cellular RNA was extracted by a single-step method (Chomczynski and Sacchi, 1987) and 1 μg of extracted RNA was converted to cDNA by reverse transcription using MoMuLV reverse transcriptase and random hexamer primers as previously described (Chi *et al.*, 1994).

Quantitative RT-PCR analysis

Our approach to quantitative RT-PCR was previously reported (Chi *et al.*, 1997). Briefly, serial cDNA dilution experiments combined with increasing PCR cycle numbers (20-38 cycles) determined that 1 : 4 diluted cDNA (12.5-25 ng/50 μl PCR reaction) undergoing 26-34 cycles was within the logarithmic phase of amplification and yielded reproducible results with all primers used for TGF-β1, TGF-β1 receptor type I and II, Smad1-Smad7, PAI-1, Fas, Fadd, Fap-1, Bcl-2, Bax, Bcl-X_L, PTEN/MMAC1, c-Fos, c-Jun, c-Myc, $p21^{Waf1}$, $p15^{Ink4b}$, $p27^{Kip1}$, and an endogenous expression standard gene GAPDH. The sequences of oligonucleotide primers used in this study are available upon request. Based on this observation, 2 μl of 1 : 4 diluted cDNA were subjected to PCR amplification with exon-specific primers for 30-34 cycles at 95°C for denaturation (1 min), 58-63°C for annealing (45 sec), and 72°C for extension (1 min) in 1.5 mM $MgCl_2$-containing reaction buffer (PCR buffer II, Perkin Elmer). Ten μl of the PCR products were resolved on 2% agarose gels and quantitation of expression levels was performed by densitometric scanning of the ethidium bromide-stained gels. Absolute area integrations of

the curves representing each specimen were compared after adjustment for the housekeeping gene (GAPDH) expression. Integration and analysis of expression levels were performed using the Molecular Analysist 2.0 software program (Bio-Rad, Hercules, CA). Quantitative RT-PCR was repeated at least 3 times and the means were calculated.

Results

Resistance to TGF-β1-mediated growth inhibition

To investigate the effect of TGF-β1 on cellular proliferation of the U937 cells, the growth of the cells was examined using the hemocytometer at 24, 48, 72 h after treatment with various concentration of TGF-β1 (1, 2, 4, and 10 ng/ml). A representative example of the assay with 4 ng/ml of TGF-β1 is shown in Figure 1. No detectable effect of TGF-β1 on cell growth of the U937 cells was observed over the range of concentrations from three independent experiments. A possible contribution of serum components on the TGF-β1 regulation of cell proliferation, was examined by lowering the level of serum to 1/10 during the assays. Almost the same results were consistently obtained (Figure 1). Flow cytometric analysis of the cell cycle also demonstrated that TGF-β1 does not affect the G_1-S or S-G_2/M transition of the cell cycle (Table 1). Taken together, our results indicate that the U937 cells is resistant to the growth inhibition mediated by TGF-β1.

Mutational characterization of TGF-β1 signaling pathway

Cellular resistance to TGF-β1-induced growth inhibition has been frequently observed in various types of human cancers including leukemia (Testa *et al*., 1993; Douglas *et al*., 1997). Previous studies demonstrated that mutational alteration of TGF-β1 receptors or intracellular mediators of TGF-β1 signaling cascade such as Smad family members is a predominant cause for the tumor resistance to TGF-β1. Thus, we characterized the expression and mutational status of the genes in TGF-β1 signaling pathway. Quantitative RT-PCR analysis revealed that mRNA expressions of TGF-β1, TβR-I, TβR-II, and 7 Smad members including Smad2, Smad3, and Smad4, which are critical intracellular mediators of TGF-β1 signal, were easily detectable in this cell line (Figure 2A). In addition, RT-PCR-SSCP analysis showed no migration shift of single-stranded DNA molecules indicating sequence substitution in the entire coding region of TβR-I, TβR-II, and Smad2-4 transcripts (Figure 2B). These results indicate that the resistance of the

Table 1. Flow cytometric analysis of TGF-β1 effect (4 ng/ml, 72 h) on cell cycle progression of the U937 cells

	G_0/G_1	S	G_2/M
10% serum			
Control	63.1 ± 5.2	17.7 ± 2.1	19.2 ± 1.6
TGF-β1	64.6 ± 4.7	18.9 ± 2.4	16.5 ± 1.8
1% serum			
Control	64.9 ± 4.7	18.0 ± 1.9	17.1 ± 1.7
TGF-β1	62.2 ± 4.8	17.6 ± 1.5	20.2 ± 2.1

The numbers mean the percentages of cells. Three independent experiments were performed and the calculated means and standard deviations (SD) were obtained.

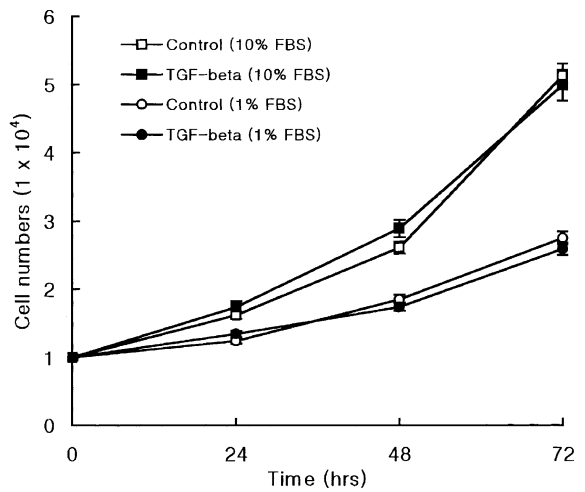

Figure 1. The effect of TGF-β1 on the proliferation of human monocytic leukemia U937 cells. The cells were plated at an initial density of 1×10^4 and treated with TGF-β1 at 4 ng/ml in the presence of 10% or 1% FBS. Cell numbers were counted every 24 h using a hemocytometer.

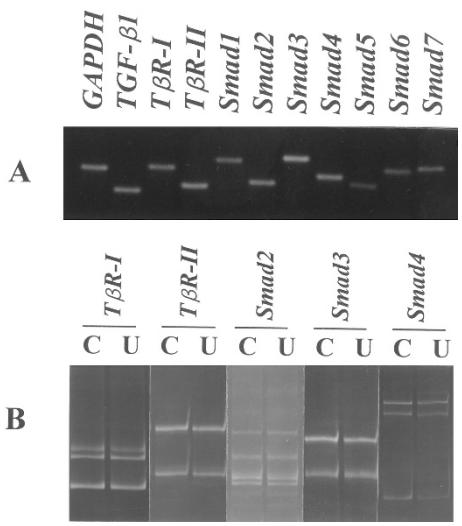

Figure 2. Expression and mutational analysis of TGF-β1 signaling pathway in U937. (A) Expression of TGF-β1, TβR-I, TβR-II, and Smad family members (Smad1-7) was examined by RT-PCR. Ten μl of RT-PCR products was resolved on a 2% agarose gel and stained with ethidium bromide. (B) Representative examples of nonisotopic RT-PCR-SSCP analysis of TβR-I, TβR-II, Smad2, Smad3, and Smad4. C, wild-type control (normal lymphocytes); U, U937.

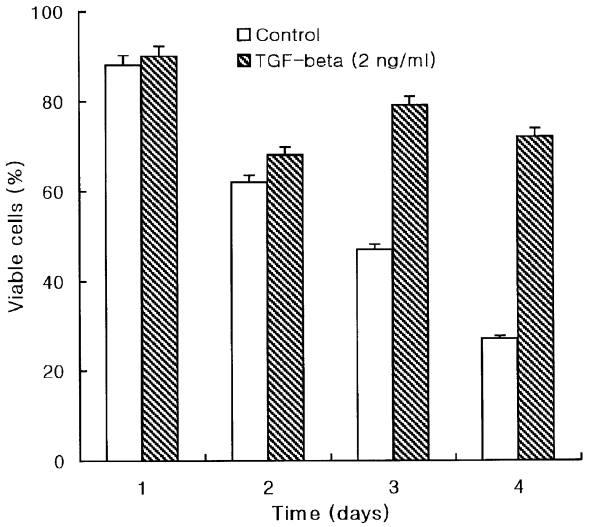

Figure 3. TGF-β1 effect on the viability of the U937 cells. 5×10^5 cells were cultured in serum-deprived medium for 4 days in the absence or presence of TGF-β1 (2 ng/ml). Viable cells were discriminated by trypan blue exclusion and counted at every 24 h using a hemocytometer.

U937 cells to TGF-β1 might be caused by other factor(s) rather than the mutational alteration of the genes in TGF-β1 signaling pathway.

TGF-β1 enhances cellular viability

In addition to the regulation of cell proliferation, TGF-β1 has been observed to affect various biological processes through multiple routes. To explore whether the cellular viability or apoptotic death of the U937 cells is affected by TGF-β1, we initially examined the effect of TGF-β1 on the viability of cells under growth factor-deprived culture condition. Trypan blue exclusion as-say demonstrated that the number of viable cells is significantly increased by TGF-β1 (2 ng/ml) treatment in comparison with the non-treated cells. As shown in Figure 3, approximately 70-73% of cells were viable in TGF-β1-treated group while only 25-33% of control cells were viable at day 4 after growth factor-deprivation. Such finding suggests that TGF-β1 might act as a viability factor for this leukemia cell line and thus provide the cells a survival advantage under the certain unfavorable physiological conditions. Flow cytometric analysis also demonstrated that growth factor deprivation-induced apoptosis is markedly inhibited by TGF-β1 treatment (Table 2). In three independent flow cytometric assays, only 11.9% of TGF-β1 (2 ng/ml, 72 h)-treated cells were detected within hypodiploid peak indicating apop-totic cell death, while 73.4% of untreated cells showed apoptosis, suggesting that the viability enhancing effect of TGF-β1 might be mediated by an inhibition of apoptotic cell death.

Inhibition of Fas- and DNA damage-induced apoptosis by TGF-β1

Table 2. Flow cytometric analysis (sub G_1) of TGF-β1 effect (2 ng/ml) on apoptotic cell death of the U937 cells

	Serum starvation (96 hrs)	Fas antibody (100 ng/ml, 16 hrs)	Etoposide (100 μM, 8 hrs)
Control	73.4 ± 9.3	36.4 ± 5.4	49.2 ± 4.6
TGF-β1	11.9 ± 5.7	9.8 ± 1.8	19.1 ± 3.2
Anti-TGF Ab	69.4 ± 4.7	37.9 ± 4.1	47.5 ± 3.8

The numbers mean the percentages of hypodiploid peaks. Three independent experiments were performed and the calculated means and standard deviations (SD) were obtained.

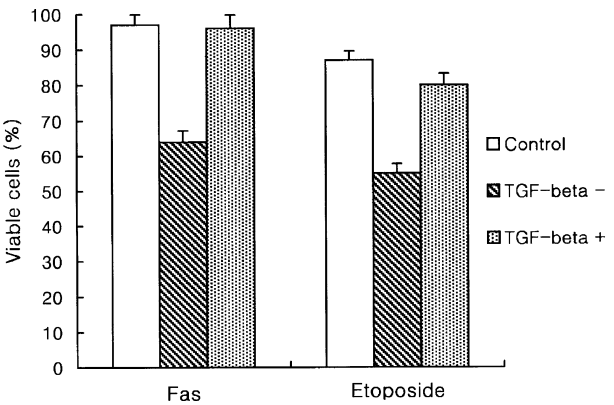

Figure 4. Effect of TGF-β1 on Fas- or DNA damage-induced apoptosis of the U937 cells. 5×10^5 cells were pretreated with TGF-β1 (2 ng/ml) for 48 h, and 100 ng/ml of anti-Fas antibody or 100 μM of etoposide was treated for 24 or 8 h, respectively. Viable cells were discriminated by trypan blue exclusion and counted using a hemocytometer.

The fact that TGF-β1 was unable to affect cell proliferation but able to enhance cell survival raised the possibility that TGF-β1 might regulate the cellular sensitivity to apoptotic stimuli. The effects of TGF-β1 on Fas- and DNA damage-induced apoptosis of the U937 cells was determined. Trypan blue exclusion assay revealed a marked reduction of viable cells following treatment with anti-Fas antibody (100 ng/ml). As shown in Figure 4, approximately 36% of the cells showed apoptotic death at 16 h after anti-Fas antibody treatment. However, a significant inhibition of the Fas-mediated apoptosis was detected by pretreatment of the cells with TGF-β1. The TGF-β1 inhibition of Fas-mediated apoptosis occurred in a time- and dose-dependent manner (Figure 5A). Flow cytometric analysis also demonstrated a significant reduction of the hypodiploid peak in TGF-β1-pretreated cells (Figure 5B). Moreover, the TGF-β1 inhibition of Fas-mediated apop-tosis was completely abrogated by co-treatment of the cells with anti-TGF-β1 neutralizing antibody (2 μg/ml), indicating the TGF-β1 specificity of this antiapoptotic effect (Table 2). To explore that TGF-β1 can also inhibit apoptosis induced by other apoptosis-triggering stimuli such as DNA damage, we treated the cells with a DNA damage-inducing chemotherapeutic agent, etoposide, and the TGF-β1 effect on the induction

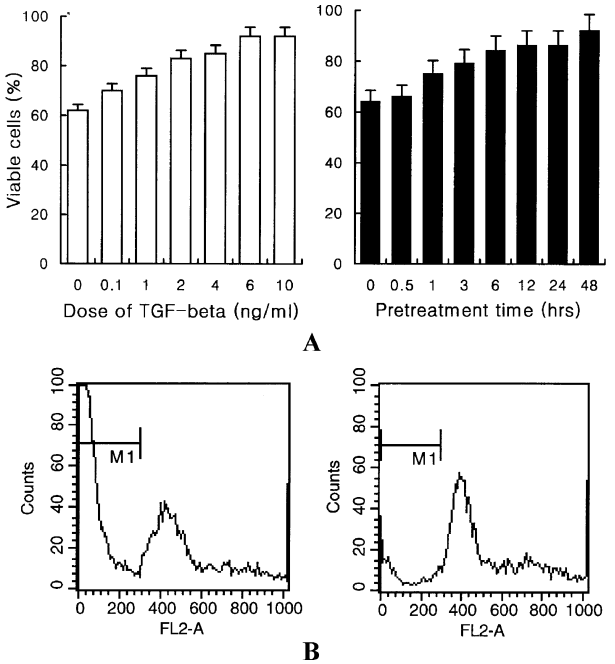

Figure 5. (A) Time and dosage-dependent inhibition of Fas-mediated apoptosis by TGF-β1. 5×10^5 cells were pretreated with various concentration of TGF-β1 (0.1-10 ng/ml) for 24 h (left) or the cells were pretreated with 2 ng/ml of TGF-β1 for various time (0.5-48 h) (right). The cells were then treated with 100 ng/ml of anti-Fas antibody for 24 h and viable cells were discriminated by trypan blue exclusion. (B) Apoptosis induction by anti-Fas antibody (100 ng/ml, 24 h) with (right) or without (left) pretreatment of TGF-β1 (2 ng/ml, 48 h). The hypodiploid peak indicating apoptotic death (M1) was examined using FACScan flow cytometry.

of apoptosis was analyzed. Close to one half of the cells (44%) showed apoptotic death at 8 h following the etoposide treatment (100 μM), but only 20% of the cells were identified to show apoptosis in the TGF-β1-pretreated group (Figure 4). Considering that 10-15% of the control U937 cells showed spontaneous apoptosis in our culture condition, our observation suggests that a substantial fraction of the DNA-damaged cells could escape from apoptotic death by TGF-β1 pretreatment (Table 2).

Flow cytometric analysis of CD14

Monocytic differentiation of the U937 cells by several differentiation-inducing factors such as TPA has been previously demonstrated (Keller *et al*., 1991; Testa *et al*., 1993). To define that inhibition of Fas- or DNA damage-mediated apoptosis is associated with differentiation of the cells by TGF-β1, we performed a flow cytometric analysis of surface expression of CD14, a marker for monocytic differentiation (Hamon *et al*., 1994). Using a FITC-conjugated CD14 antibody, we examined the expression of CD14 following TGF-β1 treatment, but no difference in expression level was observed between treated and untreated cells (Figure 6).

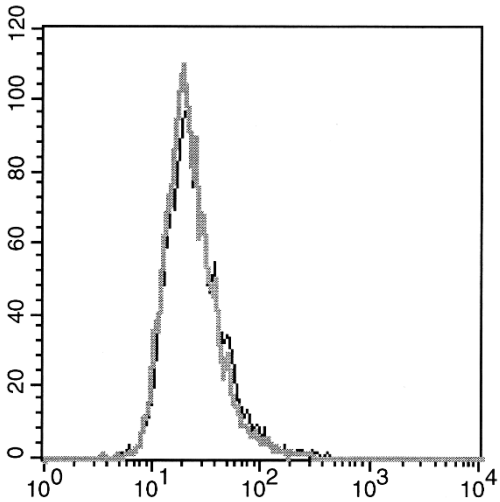

Figure 6. Analysis of CD14 expression in TGF-β1-treated U937 cells by flow cytometry. Expression of CD14 was examined TGF-β1 (2 ng/ml)-treated (gray line) and control (black line) cells using FITC conjugated anti-CD14 antibody and FITC conjugated goat anti-mouse IgG as control. The cells were analyzed by a FACScan flow cytometry. X-axis, flurorescence intensity; Y-axis, cell numbers.

Transcriptional induction of Bcl-X_L by TGF-β1

TGF-β1 exerts its function through the transcriptional regulation of cellular target genes (Derynck and Zhang, 1996; Massague, 1996). To define whether the anti-apoptotic effect of TGF-β1 is associated with its regulatory function of gene expression, we analyzed expression of the genes which have been known to play important roles in the apoptosis signaling pathway using a quantitative RT-PCR analysis. As shown in Figure 7, increased mRNA expression of a representative TGF-β1 responsive gene, plasminogen activator inhibitor-1 (PAI-1), whose transcriptional up-regulation has been well described in several other cell lines, was observed following treatment. In addition, a time-dependent transcriptional induction of other TGF-β1 responsive genes such as TGF-β1 itself and TβR-II was detected, indicating that the signaling cascade of TGF-β1-mediated transcriptional regulation is intact in this cell line. To explore whether the transcription of the genes involved in Fas-mediated apoptosis pathway is affected by TGF-β1, we analyzed expressions of Fas, Fadd, and Fap-1, but no detectable changes in mRNA levels of these genes were recognized. We next examined expression of intracellular apoptosis effector genes that are commonly involved in various apoptosis signaling pathways such as Caspase-3 (Cpp32), Bcl-2, Bcl-X_L, and Bax. Interestingly, we found a significant induction of Bcl-X_L by TGF-β1 while no changes were detected in the expression of Bcl-2, Bax, and Caspase-3. The transcriptional up-regulation of Bcl-X_L by TGF-β1 was recognized in a time-dependent fashion (Figure 7). Thus, our results strongly suggest that the antiapoptotic function of TGF-β1 might be mediated by its stimulatory effect on

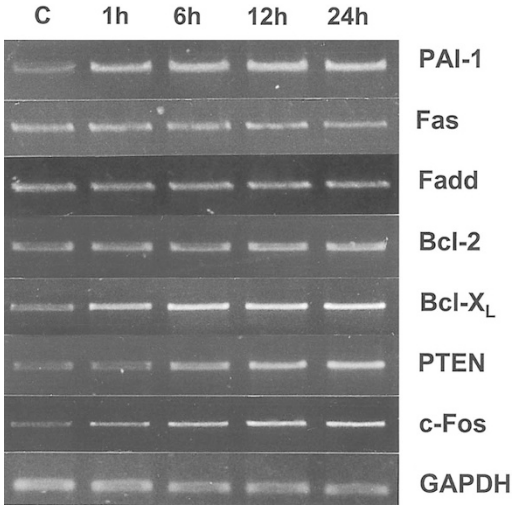

Figure 7. Quantitative RT-PCR analysis of TGF-β1 regulation of gene expression. The U937 cells were treated with TGF-β1 (2 ng/ml) for various periods of time. PCR was performed with exon-specific primers and 10 μl of the PCR products were resolved on a 2% agarose gel. GAPDH was used as an endogenous expression standard for quantitation. C, control.

the transcription of an apoptosis inhibitor gene, Bcl-X_L.

Induction of the PTEN/MMAC1 tumor suppressor gene

It has been demonstrated that TGF-β1 induces Cdk inhibitors such as $p21^{Waf1/Cip1}$, $p15^{Ink4b}$, and $p27^{Kip1}$, and they are critical effectors of the TGF-β1-mediated inhibition of cell proliferation and tumor growth (Hannon and Beach, 1994; Polyak *et al*., 1994; Datto *et al*., 1995). We examined expressions of the Cdk inhibitors and several other cell cycle-controling genes. Consistent with our results of cell cycle analysis, TGF-β1 treatment neither affect the transcription of Cdk inhibitors $p21^{Waf1/Cip1}$, $p15^{Ink4b}$, and $p27^{Kip1}$ or affect positive cell cycle regulators Cyclin D1, Cdc2, c-Myc, and c-Jun. However, an increased expression of c-Fos was observed. In addition, dose- and time-dependent induction of PTEN/MMAC1, a recently identified tumor suppressor gene, was observed following TGF-β1 treat-ment (Figure 5). TGF-β1 induction of PTEN/MMAC1 transcription was detectable at 6 h and continued to 24 h after treatment.

Discussion

The results of this study show that TGF-β1 acts as a cell viability factor in human monocytic leukemia U937 cells via inhibition of apoptotic cell death. Although cell cycle progression or differentiation was not affected, apoptotic cell death induced by growth factor deprivation, Fas antibody, or Etoposide, was significantly inhibited by TGF-β1 and this inhibition is completely abrogated by co-treatment of a neutralizing anti-TGF-β1 antibody, indicating the TGF-β1 specificity of this antiapoptotic effect. A previous study demonstrated that the tumor suppressor, p53 can mediate TGF-β1-induced differentiation of leukemia cells (Ehinger *et al*., 1997). Possible alteration of p53 gene in U937 cells may have caused incapacitation of the cells to proliferate and differentiate upon TGF-β1 treatment, is an intriguing idea to be confirmed. However, the TGF-β1 effect on cell proliferation of the U937 cells is controversial. Lastres (1996) found that proliferation of the cells is decreased in the presence of TGF-β1, but Testa *et al*, (1993) found that proliferation of U937 is essentially unaffected by TGF-β1 whereas cell growth is inhibited by combined treatment with vitamin D3 or dexamethasone. The reason for this discrepancy on growth regulation of TGF-β1 is not clear. Pluralistic effect of TGF-β1 has also been found in macrophage proliferation; it acts as a negative regulator in bone marrow, but enhanced proliferation in tissues (Celada and Maki, 1992), TGF-β1 also regulated cell growth, positively or negatively depending on the cell context (Keller *et al*., 1991). Treatment of the TGF-β1 treated U937 cells with an c-Fos antisense oligomer did not affect the growth (Kanatani *et al*., 1996). This report is consistent with our observation of TGF-β1 stimulation of c-Fos but no growth inhibition of the cells by this cytokine.

TGF-β1 has profound apoptosis-inducing effects on certain B cell populations (Buske *et al*., 1997). In contrast, TGF-β1 was also identified to inhibit Fas-induced apoptosis of activated human T cells, suggesting that TGF-β1 might play a role as an important viability factor possibly of importance for the generation of effector and/or long-lived memory T cells (Cerwenka *et al*., 1996). TGF-β1 protection of Fas-mediated apoptosis was also observed in human rheumatoid synovial cells and murine myeloid progenitor cells (Kawakami *et al*., 1996; Dybedal *et al*., 1997). However, in solid tumors including high grade glioma, TGF-β1 was observed to enhance Fas-mediated apoptosis (Ashley *et al*., 1998). Taken together, these observations indicated that TGF-β1 functions as a bidirectional regulator of apoptosis. Here we report that Fas-mediated apoptosis of human monoblastic myelogenous leukemia cells U937 is inhibited by TGF-β1. The antiapoptotic effect was not specific for Fas-mediated apoptosis but was observed for DNA damage- or growth factor deprivation-induced apoptosis.

The diverse biological actions of TGF-β1 stem from its capability to regulate the transcription of specific set of target genes. Recently, TGF-β1-induced cell cycle arrest was found to be partially attributed to the regulatory effects of TGF-β1 on the transcription of Cdk inhibitors such as $p21^{Waf1}$, $p15^{Ink4b}$, or $p27^{Kip1}$ (Hannon and Beach, 1994; Polyak *et al*., 1994; Datto *et al*., 1995). Our quantitative RT-PCR analysis showed that mRNA expression of TGF-β1 responsive genes including PAI-1 and TβR-II is significantly up-regulated by TGF-β1 treatment, indicating that the TGF-β1 signaling cascade

for transcriptional regulation is intact in the U937 cells we examined. Interestingly, we observed the transcriptional up-regulation of Bcl-X_L, a potent inhibitor of apoptosis, by TGF-β1 while no changes in expression levels of Fas, Fadd, Fap-1, Bcl-2, Bax, and Caspase-3 (Cpp32) were found. The dose- and time-dependent character of both apoptosis inhibition and Bcl-X_L induction by TGF-β1 suggests that the transcriptional up-regulation of Bcl-X_L might play an important role in TGF-β1-mediated inhibition of apoptosis. A previous study also showed that expression of Bcl-X_L correlates with survival of hemopoietic cells along the monocyte/macrophage lineage (Chatterjee *et al*., 1997). Thus, our result strongly indicates that the inhibitory effect of TGF-β1 on apoptosis might be mediated by its transcriptional up-regulation of Bcl-X_L. Consistent with our results of the cell cycle analysis, no changes in expression levels of Cdk inhibitors $p21^{Waf1/Cip1}$, $p15^{Ink4b}$, or $p27^{Kip1}$ and positive cell cycle regulators Cyclin D1, Cdc2, or c-Jun were identified.

PTEN/MMAC1 is a newly identified tumor suppressor gene whose product has a extensive homology with tensin that appears to bind actin filaments at focal adhesions (Li *et al*., 1997a). It was observed that overexpression of PTEN/MMAC1 inhibits cell migration, whereas antisense PTEN/MMAC1 enhances migration (Tamura *et al*., 1998). Recently, rapid transcriptional down-regulation of PTEN/MMAC1 by TGF-β1 was found in actively growing human keratinocyte cells, HaCaT (Li *et al*., 1997b). However, we observed an unexpected dose- and time-dependent induction of PTEN/MMAC1 in TGF-β1-treated U937 cells. Significance of the TGF-β1 induction of the PTEN/MMAC1 tumor suppressor and an association with its anti-apoptotic effect observed in this study is not yet understood and molecular analysis of the PTEN/MMAC1 role in TGF-β1 signaling pathway is currently in progress.

In conclusion, our data show that TGF-β1 increases cellular resistance of human monoblastic leukemia U937 cells to apoptotic cell death which is triggered by various signals including Fas. Our study also showed that the antiapoptotic effect of TGF-β1 is associated with its transcriptional up-regulation of an apoptosis inhibitor Bcl-X_L. Therefore, this study strongly suggests that the antiapoptotic effect of TGF-β1 may provide tumor cells a survival advantage and thus contribute to the malignant progression of human leukemic cells.

Acknowledgments

This work was supported in part by research grants from Ministry of Education, Korea 1998.

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