0026-895X/03/6402-415–420$7.00
MOLECULAR PHARMACOLOGY
Vol. 64, No. 2
2313/1082503
Printed in U.S.A.

Mol Pharmacol 64:415–420, 2003

Thalidomide Down-Regulates Transcript Levels of GC-Rich Promoter Genes in Multiple Myeloma

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Received December 3, 2002; accepted May 9, 2003

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

Thalidomide (Thd), a potent teratogen, was shown to have therapeutic potential in cancer, primarily in multiple myeloma (MM), yet its mechanism of action has not been elucidated. It was recently suggested that its teratogenicity is derived from interference in expression of genes regulated by GC-rich promoters by blocking the binding of SP1 transcription factor to its motif. We explored the validation of the proposed model by focusing on potential molecular targets associated with MM pathogenesis. Cell lines RPMI 8226, U266, and ARH-77 were exposed for 24 h to racemic Thd and analyzed for apoptosis, membranal expression of CD29 and CD63, transcript level of hTERT, CD63, and IGFI-R (characterized by GC-rich motifs) and telomerase activity. Analysis of an hTERT core promoter reporter gene expression [enhanced green fluorescent protein (EGFP)] in transiently transfected RPMI 8226 incubated with racemic and steric (±)-enantiomers of Thd was performed. A consistent reduction (~10–40%) in transcript levels of all three assayed genes in all three cell lines was demonstrated in the presence of racemic Thd. Significant reduction of EGFP was demonstrated in cells transfected with hTERT reporter gene and treated with racemic and (*S*)-Thd. Our results show that Thd's antimyeloma activity can be ascribed to the same mechanism responsible for its teratogenic effect and that the inhibition of GC-rich promoter genes is mostly attributed to the *S*-racemate. Indeed, this selectivity delineates GC-rich promoter genes as a unique group eligible for specific drug targeting.

Thalidomide (Thd), a potent teratogen, has been found to have antineoplastic activity in multiple myeloma (MM) (Noopur, 1999). MM is an incurable plasma cell neoplasia predominantly localized in the bone marrow. The clinical manifestations include bone lesions, renal damage, pancytopenia, hypercalcemia, and recurrent infections. The disease is incurable and progresses despite currently available treatment, including high-dose chemotherapy with stem cell support (Noopur, 1999; Singhal et al., 1999). The malignant clone is characterized by intensive interactions with bone marrow stroma modulated by membranal embedded components (i.e., cytokines, growth-factors, adhesion and signaling molecules) (Epstein and Yaccoby 2002). The initial rationale for the use of Thd in myeloma was its antiangiogenic potential, but accumulating data indicate that it may act through other mechanisms as well. Stephens et al. (2000) have proposed a model explaining Thd's mechanism of action that unifies more than 30 hypotheses. The model stems from the previously demonstrated Thd inhibition of the IGF-I and fibroblast growth factor-2 pathways necessary for limb bud formation. Both pathways encompass multiple genes with promoters lacking TATA and CAAT boxes and characterized by G-rich domains. Thd has a chiral center and is commonly used as a racemate (1:1 mixture) of its stereochemical *R*- and *S*-structures. Based on computational modeling that determines that *S*(−)-Thd (but not *R*(+)-Thd) is able to intercalate into the major groove of DNA in G-rich domains, it was suggested that by blocking promoter binding of SP1 and AP1 transcription factors the expression of genes lacking TATA and CAAT boxes may be down-regulated.

Genes characterized by GC-rich promoters control some pivotal cell-to-cell and cell-to-extracellular matrix interactions. Included are integrins (CD29); tetraspanins (CD63), and IGF-IR (Cooke et al., 1991; Hotta et al., 1992; Villa-Garcia et al., 1994). The promoter of hTERT (the catalytic component of telomerase) is also GC-rich and contains in its core sequence five SP1 recognition sites previously demonstrated to be fundamental to its regulation (Takakura et al., 1999). Elevated levels of telomerase activity have been demonstrated in most neoplasias, including MM (Nilsson et al., 1994; Shiratsuchi et al., 1999), and transcriptional regulation of the hTERT subunit is conceived as the limiting step in telomerase expression (Takakura et al., 1999).

This study was designed to validate the specific modula-

ABBREVIATIONS: Thd, thalidomide; MM, multiple myeloma; IGF, insulin-like growth factor; IGF-IR, insulin-like growth factor I receptor; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; FACS, fluorescence activated cell sorting; PCR, polymerase chain reaction; APC, adenomatous polyposis coli; TRAP, telomeric repeat amplification protocol; RT-PCR, reverse transcription-polymerase chain reaction.

tion of GC-rich promoter genes by Thd. We chose MM as the research model based on its relative sensitivity to Thd treatment (response of 30% of the chemotherapy-resistant patients) and the absence of a comprehensive mechanistic understanding (D'Amato et al., 2001; Neben et al., 2001; Rajkumar, 2001; Richardson et al., 2002). The choice target genes are regulated by GC-rich promoters and may be associated with the pathogenesis of MM. Establishment of an association between Thd and specific molecular targets may facilitate rational design of potent analogs targeting specific pathways while avoiding adverse effects. It may also have therapeutic implications in MM.

Materials and Methods

Materials. All culture mediums and supplements were purchased from Biological Industries (Beit Haemek, Israel). (±)-Thalidomide was purchased from Calbiochem (La Jolla, CA); *R*(+)- and *S*(−)-Thalidomide were purchased from Sigma (St Louis, MO); TRAPeze telomerase detection kit was a product of Serologicals Corp. (Norcross, GA) and Syber Green I was also from Sigma.

TABLE 1
Primers

β-Actin	
Forward	5′-GAC CAC ACC CCT CGT AGA TGG G-3′
Reverse	5′-GCA TAC CCC TCG TAG ATG GG-3′
CD63	
Forward	5′-CCC GAA AAA CAA CCA CAC TGC-3′
Reverse	5′-GAT GAG GAG GCT GAG GAG ACC-3′
IGF-IR	
Forward	5′-CCA TCA GGA AGT ATG CCG AC-3′
Reverse	5′-CGC TGA TCC TCA ACT TGT G-3′
hTERT	
Forward	5′-TGA ACT TGC GGA AGA CAG TGG-3′
Reverse	5′-ACA TGC GTG AAA CCT GTA CG-3′
APC	
Forward	5′-TCA ATA CCC AGC CGA CCT-3′
Reverse	5′-CCT AGT TCC AAT CTT TTC TTT TA-3′

Cell Cultures. MM cell lines U266 and RPMI 8226, purchased from the American Type Culture Collection (Manassas, VA), were cultured in RPMI 1640 supplemented with 20% heat inactivated fetal calf serum (FCS) and antibiotics. EBV transformed plasma cell leukemia cell line ARH-77 (kindly provided by Prof. Ben-Basat, Sheaba Medical Center, Tel-Hashomer, Israel) was sustained in media containing 20% non–heat-inactivated FCS. Twenty-four hours before the experiments, 3 million cells were seeded in 10 ml of fresh media. Thd was administered to cells, and its solvent dimethyl sulfoxide was added to the control samples (0.5%). The study included four experiments conducted in duplicates assayed simultaneously for all evaluated parameters. All cell lines will be referred to from here on as "MM cell lines" without distinction.

Antibodies. Fluorescein isothiocyanate (FITC)-coupled Annexin V was purchased from Roche Applied Science. FITC-coupled monoclonal mouse anti-human CD29 and CD63 and isotype were purchased from Caltag Laboratories (Burlingame, CA).

Flow Cytometry. Fluorescence was analyzed by a Coulter flow cytometer (EPICS-XL; Beckman Coulter UK). All results are expressed as MFI and at least 10000 events were counted in each FACS analysis.

Analysis of Apoptosis. Annexin V was employed for exposed phosphatidyl serine detection according to manufacturers' instructions supplemented with 0.1 μg of propidium iodide and assayed for fluorescence by FACS. Calibration of FACS analysis parameters was designed to exclude autofluorescence assessed according to the untreated control cells (less than 5% Anx^{+}propidium $iodide^{-}$ cells). A second approach for verification of apoptosis was analysis of differential cell sizes and refractive/reflective properties established according to forward scatter and side scatter, which allowed discrimination of apoptotic from nonapoptotic cells (Darzynkiewicz et al.,

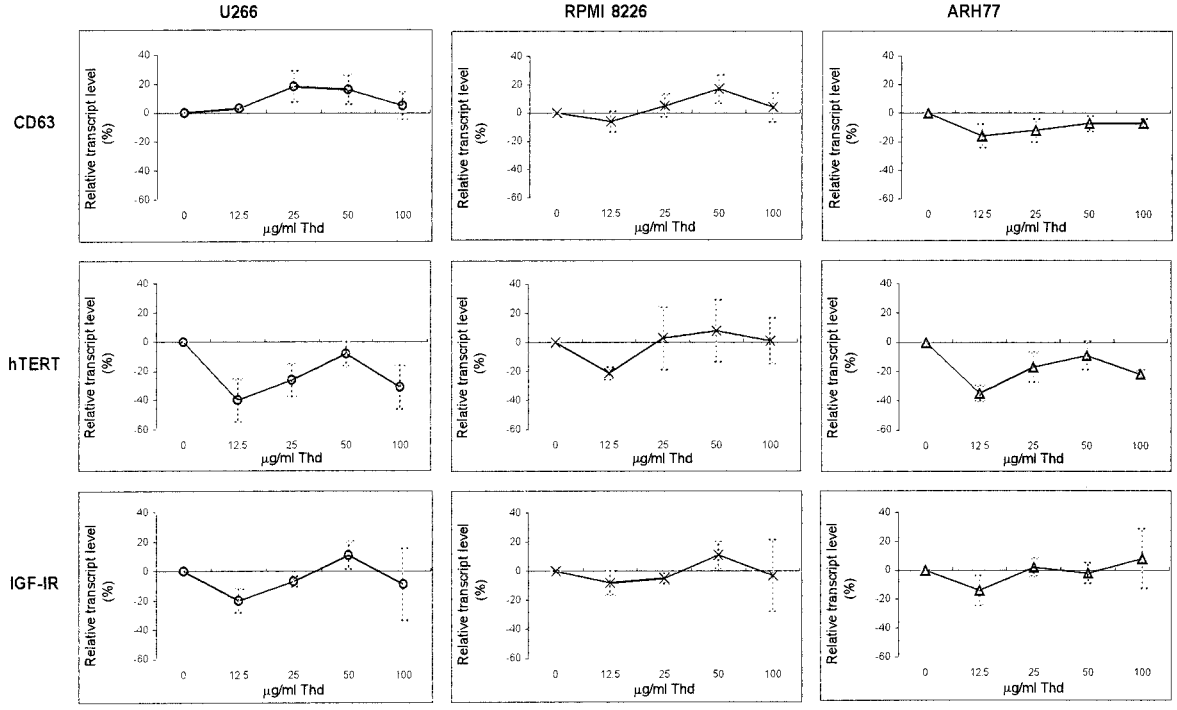

Fig. 1. Thd down-regulates transcript of target genes in MM cell lines in a dose dependent manner. The graphs depict the dose response of the respective cell lines U266, RPMI 8226, and ARH-77 (columns) to Thd treatment at the transcript level. Analysis by RT-PCR of target genes [CD63, hTERT, and IGFI-R (rows)] characterized with GC-rich promoters lacking TATA and CAAT boxes was performed. The results are expressed as the mean relative level compared with the untreated control ± S.E. The decreased mRNA levels of assayed genes in cells treated with 12.5 μg/ml racemic Thd are statistically significant in hTERT (in each cell line), IGFI-R, and CD63 (all cell lines compiled).

1992). Occasional microscopic analysis of cell morphology was used for added verification of presence or absence of apoptosis.

Determination of Surface Molecule Expression. Surface expression of CD29 and CD63 was assessed by direct immunofluorescence using mouse anti-human fluorochrome-coupled monoclonal antibodies according to manufacturer's instructions. IgG1 matched isotype was used to exclude unspecific binding.

Analysis of Green Fluorescent Protein. Fluorescence in cells transfected with reporter gene constructs was assayed at 530 nm. Mock-transfected cells were used to determine background fluorescence.

Semiquantitative RT-PCR. Total RNA was extracted from cell lines employing PURESCRIPT (Gentra Systems, Inc., Minneapolis, MN) according to manufacturer's specifications. RNA was transcribed into cDNA using SuperScript reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo $d(T)_{15}$ primers following standard procedures. Multiplex PCR of respective cDNAs and internal control was performed for CD63, IGF-IR, hTERT and adenomatous polyposis coli (APC) before and after Thd administration. PCR program was optimized at the logarithmic phase with simultaneous amplification of a housekeeping gene controlled by standard TATA and CAAT boxes containing promoter (β actin) as an internal reference of PCR efficiency. Primers are listed in Table 1. Analysis of PCR products was done employing Gel Doc 2000 (Bio-Rad Laboratories, Hercules, CA).

TRAP Assay. Telomeric repeats amplification protocol (TRAP) assay was performed with the TRAPeze detection kit according to the manufacturer's instructions. The products were separated by electrophoresis on 12.5% polyacrylamide gel and visualized with Syber Green I on Gel Doc 2000 (Bio-Rad). Activity was calculated by determining the signal intensity with the Multianalyst program (Bio-Rad).

Reporter Gene for the hTERT Core Promoter. The core promoter region of hTERT was amplified by PCR (forward, 5′-CCG TTC CGC TGG CGT CCC TGC ACC-3′; reverse, 5′-GCG GGA TCC CGC GGG GGT GGC CGG G-3′) digested with *Eco*RI and *Bam*HI, respectively, and cloned into the multicloning site of pEGFP-1 (BD Biosciences Clontech, Palo Alto, CA) upstream of the EGFP coding sequence. The construct is hereby termed 'telcore'. The plasmid was propagated using DH5α as the host strain employing standard procedures and purified employing the HiSpeed plasmid maxi kit (QIAGEN).

Transfection and Expression of Reporter Gene. Purified 'telcore' was introduced into RPMI 8226 cells by liposomal transfection with DMRIE-C (Invitrogen) according to manufacturer's instructions. In short, cells were seeded in 24-well tissue culture dishes 24 h before transfection. DNA-lipid complexes in Opti-MEM were added; 4 to 5 h later, RPMI 1640 media supplemented with FCS was added to a final concentration of 10% FCS. Thd racemic (*rac*) or either steric enantiomer (*R* or *S*) (final concentration, 12.5 or 25 μg/ml) or dimethyl sulfoxide (0.5%) were added at this point as well. Cells were cultured for 12 h and subsequently harvested and tested for EGFP fluorescence by FACS. Mock-transfected cells (treated with DMRIE-C only) were used to calibrate EGFP positive cells. Transfection efficiency was estimated according to the proportion of EGFP positive cells identified as positive for FL1 (FITC) fluorescence. Mean fluorescence in EGFP positive cells was compared between treatments and used as an indication of relative promoter utility. Multiple experiments (three or four) were conducted in triplicate. Results are expressed as mean percentage ± S.E. of relative fluoresence of Thd-treated cells compared with the EGFP-expressing cells unexposed to Thd in each respective experiment.

Statistical Analysis. Students' paired and unpaired *t*-tests were employed in analysis of differences between cohorts. An effect was considered significant when *p* value was equal or less than 0.05.

Results

Thd Down-Regulated hTERT, IGF-IR, and CD63 Transcript Levels. All RT-PCR products were normalized according to β actin amplification efficiency and then assessed compared with the transcript level of untreated cells. Mean relative transcript steady-state levels are presented in Fig. 1 and representative gels of PCR products are displayed in Fig. 2. Thd administration to all three cell lines caused a depletion of mRNA of all three assayed genes except CD63 in U266, which was up-regulated after treatment with Thd. This effect was dose-dependent with two separate peaks of repression—a prominent effect at 12.5 μg/ml and a modest one at 100 μg/ml. The dose response was demonstrated across the board in all three cell lines and genes, including CD63 in U266 cells, which displayed the most minute elevation in mRNA levels upon exposure to Thd at 12.5 μg/ml. Statistically significant reductions in transcript levels of hTERT compared with controls were demonstrated after treatment with 12.5 μg/ml Thd in all three cell lines ($p < 0.05$). The trend of reduced transcript levels compared with

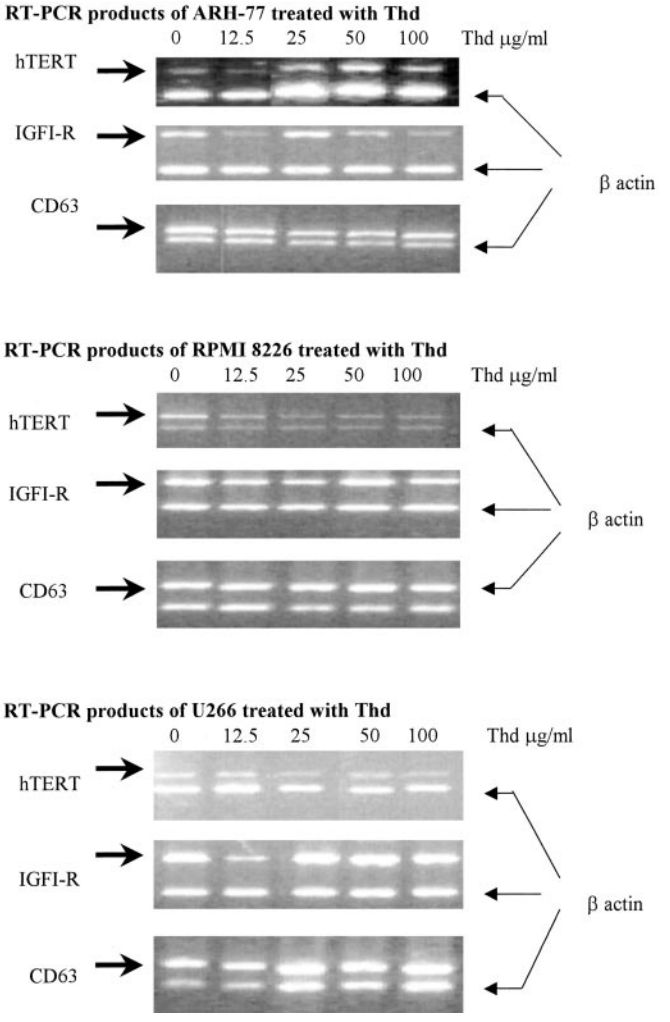

Fig. 2. Reduced hTERT, IGFI-R, and CD63 transcripts in Thd-treated MM cell lines. Representative gel electrophoresis of semiquantitative RT-PCR products is presented. Amplification of target genes from racemic Thd treated ARH-77, RPMI 8226, and U266 displayed a selective dose dependent depletion in their steady-state mRNA levels. Arrows depict the respective genes, and Thd doses are indicated at the top. Respective yield was assessed by densitometry as described under *Materials and Methods*.

untreated controls was also evident for both IGF-IR and CD63, although it did not reach statistical significance. The lower steady state transcript levels of CD63 were significantly lower compared with the elevated levels demonstrated with exposure to higher doses of Thd, which peaked at 50 μg/ml ($p < 0.05$) (Fig. 1).

Thd Did Not Deplete Transcript Level of APC. Again, RT-PCR products were normalized according to β-actin amplification efficiency and then assessed in comparison to the transcript level of untreated cells. Mean relative transcript steady state levels are presented in Fig. 3A and representative gels of PCR products are displayed in Fig. 3B. Thd administration to RPMI 8226 caused an elevation in APC mRNA levels at all assayed drug concentrations ($p < 0.05$).

Thd Did Not Induce Cell Death or Affect Telomerase Activity. Consistent with previous publications, no cell death (apoptotic or necrotic) was observed in Thd-treated MM cell lines (data not shown). Also, Thd did not affect telomerase activity in the time frame of our experiment, a finding that corresponds with the 72-h half-life of telomerase (Fig. 4).

Thd Did Not Consistently Alter Membranal Expression of CD29 and CD63. Despite changes in membranal expression levels of CD29 and CD63 displayed in Thd treated cells of all three cell lines, no constant pattern could be established. The variation in membranal level of CD63 also failed to reflect the transcript level depletion (data not shown).

Thd Inhibits Expression of hTERT Core Promoter. After establishing that Thd selectively down-regulates steady-state transcript levels of GC-rich promoter genes, we set out to assess whether this effect is mediated through the promoter sequence itself. RPMI 8226 cells were transfected with "telcore", treated with Thd, and, after 12 h, assayed for mean EGFP fluorescence per cell, indicative of promoter utilization. Uniform transfection efficiencies were displayed with approximately 30% of the cells expressing EGFP fluorescence. Exposure of transfected cells to Thd did not reduce the percentage of EGFP-expressing cells compared with controls. Diminished fluorescence, expressed as MFI of EGFP-positive cells, was demonstrated at 12.5 μg/ml (−5.5%) and 25 μg/ml (−12%) of (*rac*)Thd, both with statistical significance ($p < 0.05$ and $p < 0.005$, respectively) (Fig. 5). With the purpose of assessing the effects of Thd stereoisomers individually, we next treated transfected RPMI 8226 cells with either racemate (*R* or *S*) or the racemic mixture. Again, an inhibition of EGFP expression in treated cells was demonstrated, but to different extents. Exposure of transfected cells to *S*(−)-Thd at 12.5 and 25 μg/ml displayed 5.5 and ~12% reductions in hTERT core promoter expression, respectively ($p < 0.05$), whereas treatment with *R*(+)-Thd induced a smaller down-regulation (~4%) of EGFP expression with both doses but statistically significant at 12.5 μg/ml Thd only ($p < 0.01$) (Fig. 6). The depletion of telcore expression induced by (*rac*) as well as *S*(−)-Thd's were similar at both doses (no significant statistical difference) and the same as *R*(+)-Thd induced at a dose of 12.5 μg/ml (of all three forms of Thd). At a dose of 25 μg/ml (*R*)-Thd induced a significantly smaller reduction in EGFP fluorescence than either (*rac*) or *S*(−)-Thd ($p < 0.05$).

Discussion

The infamous Thd was recently approved by the Food and Drug Administration of the United States for use in patients with erythema nodosum leprosum (Nightingale, 1998) and is currently under evaluation for treatment of a wide variety of diseases, including malignancies. However, the mechanism of action of this drug is poorly understood. In the present study, we evaluated Thd's mode of action by investigating the validity of the hypothesis that its effect is mediated by intercalating with G-rich elements of DNA (Stephens et al., 2000).

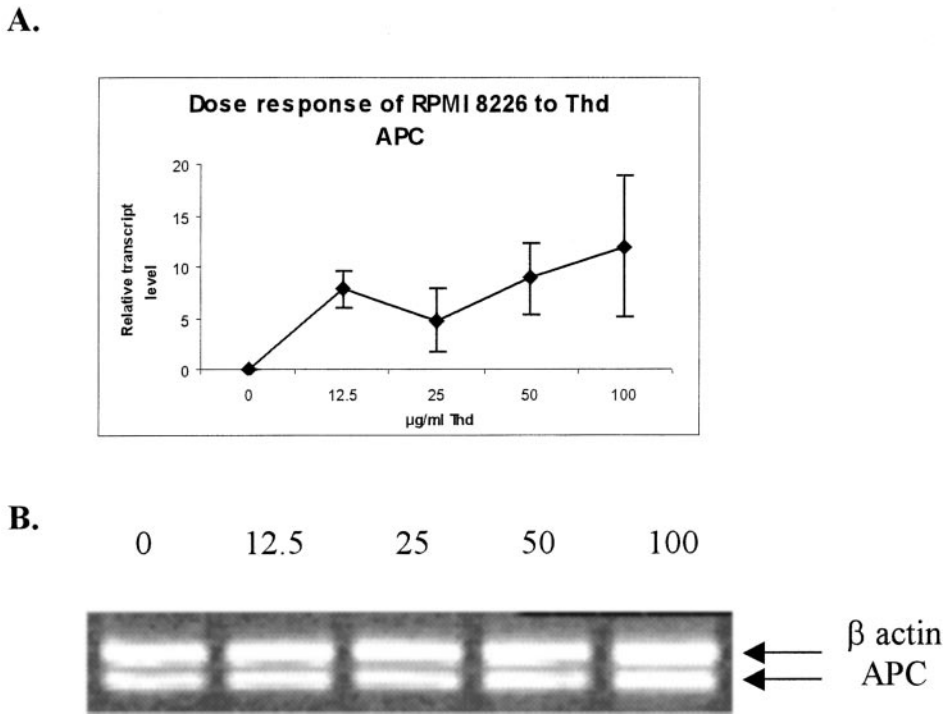

Fig. 3. APC RT-PCR products of RPMI 8226 treated with Thd. A, the graph depicts the dose response of RPMI 8226 to racemic Thd treatment at the transcript level. Analysis by RT-PCR of the target gene characterized with a non–SP1-dependent promoter was done in three separate experiments. Respective yield was assessed by densitometry as described under *Materials and Methods*. The results are expressed as the mean relative level compared with the untreated control ± S.E. The increased APC mRNA level at 12.5 μg/ml Thd was statistically significant ($p < 0.05$). B, a representative gel electrophoresis of semiquantitative RT-PCR products is presented. Amplification of APC from Thd-treated RPMI 8226 did not display the selective dose-dependent depletion in steady-state mRNA levels observed in GC-rich promoter genes. Arrows depict the respective genes and Thd doses are indicated at the top (micrograms per milliliter).

Our data provide clear evidence that Thd does inhibit transcription of specific genes with GC-rich elements by modulation of their promoter activity. Significant decreases in transcript levels of the GC-rich promoters of hTERT, IGF-IR, and CD63 were found. Furthermore, Thd directly inhibited the expression of the transfected reporter gene construct of the hTERT core promoter. These findings show that a common mechanism contributes to Thd's attenuation of both limb buds and myeloma cells.

The genes assessed in our study are GC-rich promoter targets dependent to different extents on SP1 transcription factor. Their expression was compared with that of SP1-nondependent β-actin as well as with that of the APC gene. Contrary to the constitutive expression of "house keeping" β-actin, APC is controlled by multiple factors, characterized with a CAAT box, and crucially dependent on upstream stimulating factors 1 and 2, which bind to an E-box sequence in its promoter (Jaiswal and Narayan, 2001). The APC promoter consists of a single SP1 consensus sequence, possibly contributing to p53 gene modulation, yet its necessity for p53 stimulation is undermined by the existing alternate mechanisms (i.e., direct interaction of p53 with promoter and modulation by a p53 complex with TFIIH) (Jaiswal and Narayan, 2001). Thus, APC served as an appropriate negative control in our model.

The compiled inhibitory effect of Thd on the signaling pathways of IGF-I and bFGF-2, both comprising multiple GC-rich promoter genes, is already established and its consequence is the antiangiogenic hindrance of limb bud formation culminating in its teratogenic effect (Stephens et al., 1998). We tested whether Thd inhibits key genes involved in the pathogenesis of the malignant model of MM. IGF-I activation of any given cell is mediated by its binding to an IGF-IR, the expression pattern of which is tissue-specific. The role of IGF-I pathway in MM is considered significant and fundamental to the development of the disease (Ge and Rudikoff, 2000; Qiang et al., 2002). The malignant MM cells are constantly exposed to IGF-I present in the circulation or produced by osteoclasts in the bone marrow matrix (Nilsson et al., 1999). Therefore, possible attenuation of the cell response to IGF-I may be achieved by down-regulating the expression level of its receptor. Our results of reduced levels of IGF-IR transcript not only corroborate Thd's mode of action but also suggest that it may decrease the level of both IGF-I and its receptor, thereby having a compiled effect on this molecule's activity. Similarly, we demonstrated a definite inhibition of hTERT, thereby depicting it as a relevant target for Thd modulation. hTERT is substantially elevated in a very select group of progenitor cells and is mostly characteristic of the malignant phenotype (Nilsson et al., 1994; Urquidi et al., 2000). Moreover, hTERT is regarded as one of the obligatory steps in malignant transformation (Hahn et al., 1999). Attenuating telomerase expression may not only repress the growth of cancerous cells but also allow selection of the malignant clone over most normal tissues and cells.

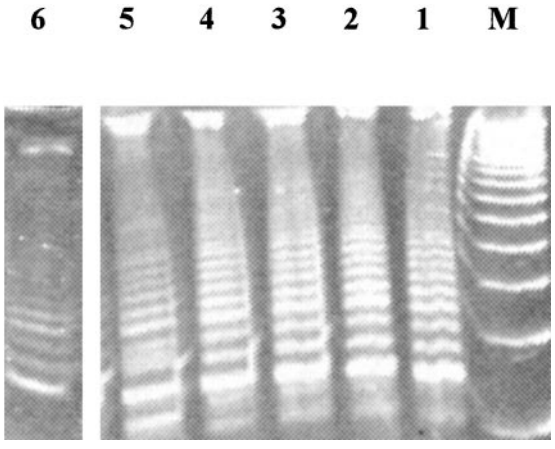

Fig. 4. Twenty-four hour incubation with Thd did not affect telomerase activity in myeloma cell lines. Representative electrophoresis gels of TRAP assay of telomerase activity are presented. Cell lysates of treated RPMI 8226 were assessed for telomerase activity according to common protocol. Analysis of respective yield by densitometry as described under *Materials and Methods* showed no significant difference in telomerase activity between paired treated and untreated cell samples. Lane M, 20-base pair molecular weight marker; lanes were treated with respective doses of Thd: lane 1, (negative control) 0 μg/ml; lane 2, 12.5 μg/ml; lane 3, 25 μg/ml; lane 4, 50 μg/ml; lane 5, 100 μg/ml; lane 6, standard TRAP assay control.

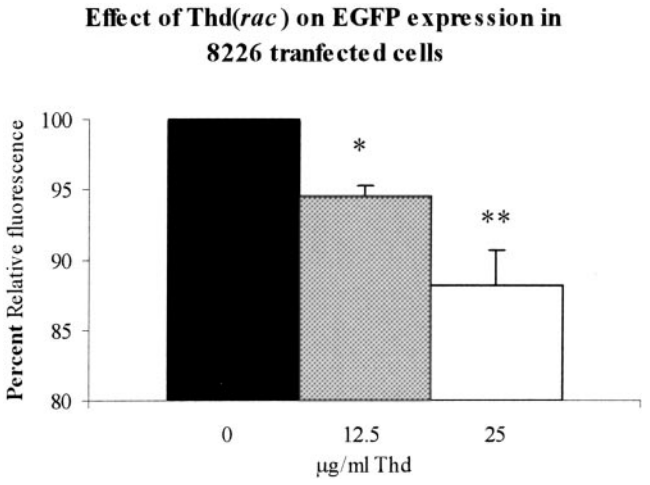

Fig. 5. Reduced EGFP fluorescence in "telcore" transfected cells treated with Thd. RPMI 8226 cells transfected with "telcore" were treated with varying doses of (*rac*)Thd. Fluorescence was assayed by FACS and expressed as percentage of EGFP fluorescence in cells untreated with Thd (mean ± S.E.). *, $p < 0.05$; **, $p < 0.01$.

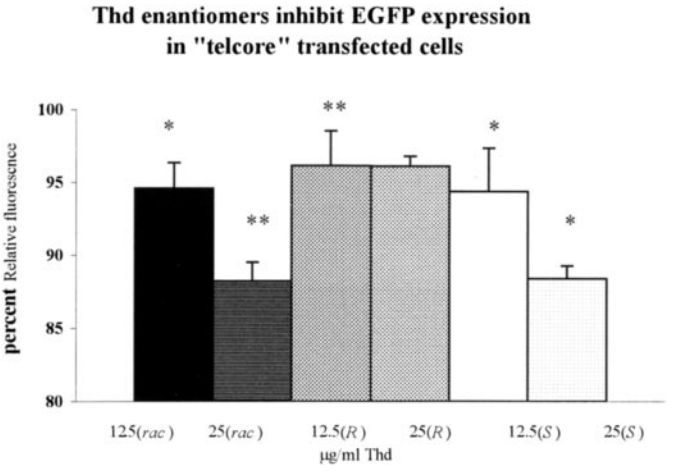

Fig. 6. (*rac*)Thd and steric enantiomers inhibit "telcore" expression in transfected cells to different extents. RPMI 8226 cells transfected with "telcore" were treated with varying doses of Thd enantiomers. Fluorescence was assayed by FACS and expressed as percentage of EGFP fluorescence in cells untreated with Thd (mean ± S.E.). (*rac*) designates the racemic Thd; (*R*) indicates the (+) enantiomer of Thd; (*S*) symbolizes the (−) enantiomer of Thd. *, $p < 0.05$; **, $p < 0.01$.

Thd's attenuation of GC-rich promoter genes was also exemplified in its selective depletion of the representative tetraspanin CD63 mRNA level. Tetraspanins in general are associated with cell survival, apoptosis, tumorigenesis, and motility (Maecker et al., 1997). More specifically, CD63 molecules present on the surface of plasma cells, may be related to serum interleukin-6 levels in patients with hematological malignancies and interleukin-6 signaling is an essential element in the bone marrow surroundings promoting myeloma cell growth (Nomura et al., 1999; Epstein and Yaccoby, 2002). The complexity of adhesion signaling as well as the overlapping with growth factor pathways most probably contributes to the less conclusive effect of Thd.

Thd, most commonly applied as a racemate of its stereochemical structures phthalimidoglutaramic acid (*S*)-Thd and carboxybenzamidoglutarimide (*R*)-Thd, undergoes in aqueous solution rapid inversion and hydrolysis (Eriksson et al., 2001). Based on Thd's affinity to guanine and computational modeling, it was suggested that the *S*-enantiomer, not the *R*, is spatially capable of incorporating into the DNA double helix, thereby interfering with normal gene function. Indeed, the major antiangiogenic activities of Thd and its teratogenicity are attributed primarily to the *S*-racemate (Eriksson et al., 2001; Thomas and Kantarjian, 2001). In our study, we assessed the relative activity of each enantiomer, and our findings support the theoretical model of the DNA intercalation of the respective racemates. Indeed, the *S*-racemate inhibited the expression of the EGFP reporter gene of the hTERT core promoter in a manner resembling that of the racemic mixture, whereas the *R*-enantiomer displayed a more limited inhibition altogether. Despite several studies displaying the need of metabolic activation of racemic Thd, we demonstrated an inhibition of gene transcription with hydrolysis products only (Kenneth et al., 1998). Similar reports regarding the effects of hydroxylation without bioactivation have been published previously (Marks et al., 2002).

Our results indicate that the reduction in transcript levels is dose-related and may be clinically relevant. These concentrations in vitro correspond to the usually high doses that are effective in myeloma in vivo (Erikkson et al., 2001; Mujagic et al., 2002; Ng et al., 2002). Pharmacodynamic and pharmacokinetic studies of Thd suggest that the bioavailability of the drug differs between specific patient groups and that the rate of absorption may be dose-dependent (Heney et al., 1991; Physicians' Desk Reference, 2000; Erikkson et al., 2001). Thus, it is possible that the particular range of Thd doses demonstrated to attenuate transcription combined with the established variability of drug absorption might explain the differential antimyeloma response to Thd treatment.

Taken together, our results demonstrate a simultaneous transcriptional inhibition of GC-rich promoter genes in myeloma cell lines. We suggest that a similar cumulative in vivo effect may contribute to Thd's antimyeloma function. These findings also imply that Thd in the different cells of myeloma and limb buds instigates the very same mechanism. Deciphering Thd's mechanism of action defines GC-rich promoters as a unique group and an eligible and specific drug target. Strategic drug combination may promote anticancer activity while avoiding dose limiting side effects.

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