

## Full-length article

**Inhibition of Bcl-2 expression by a novel tumor-specific RNA interference system increases chemosensitivity to 5-fluorouracil in HeLa cells**Sheng-lin HUANG, Yi WU, Hai YU, Ping ZHANG, Xing-qian ZHANG, Lei YING, Han-fang ZHAO<sup>2</sup>*Research Center for Human Gene Therapy, Department of Biochemistry and Molecular Biology, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China***Key words**RNA interference; telomerase; promoter regions (Genetics); *Bcl-2*; fluorouracil; gene therapy<sup>1</sup>Project supported by the Fourth Key Subject Foundation of Shanghai Municipal Education Commission (ZDXK2001).<sup>2</sup>Correspondence to Prof Han-fang ZHAO. Phn/Fax 86-21-3406-0742. E-mail zhaohf@shsmu.edu.cn

Received 2005-07-19

Accepted 2005-09-20

doi: 10.1111/j.1745-7254.2006.00247.x

**Abstract**

**Aim:** RNA interference (RNAi) has been proposed as a potential treatment for cancer, but the lack of cellular targets limits its use in cancer gene therapy. No current technology has achieved direct tumor-specific gene silencing using RNAi. In the present study we attempt to develop a tumor-specific RNAi system using the human telomerase reverse transcriptase (hTERT) promoter; furthermore, we analyzed its inhibitive effect on *Bcl-2* expression. **Methods:** The vectors containing a small hairpin RNA (shRNA) to target exogenous reporters [firefly luciferase and enhanced green fluorescent protein (*EGFP*)] and endogenous gene (*Bcl-2*) were constructed. Luciferase expression was determined by dual luciferase assay. Reverse transcription-polymerase chain reaction (RT-PCR), fluorescence microscopy and fluorescence-activated cell sorting (FACS) were used to measure *EGFP* expression. Inhibition of *Bcl-2* was evaluated by RT-PCR and Western blotting. Cell proliferation and viability were measured by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. FACS was used to analyze the cell cycle distribution profile. **Results:** We showed that with the hTERT promoter directly driving shRNA transcription, expression of the exogenous reporters (*LUC* and *EGFP*) in tumor cells, but not normal cells, was specifically inhibited *in vitro*. The hTERT promoter-driven shRNA also depressed the expression of *Bcl-2*. Inhibition of *Bcl-2* did not affect cell proliferation, but increased the chemosensitivity of HeLa cells to 5-fluorouracil. **Conclusion:** The present study describes an efficient RNAi system for gene silencing that is specific to tumor cells using the hTERT promoter. Suppression of *Bcl-2* by using this system sensitized HeLa cells to 5-fluorouracil. This system may be useful for RNAi therapy.

**Introduction**

RNA interference (RNAi) is a sequence-specific post-transcriptional gene-silencing process that has been proposed as a potential treatment for cancer<sup>[1]</sup>. Specific gene silencing can be achieved in a variety of cell systems using chemically synthesized siRNA or DNA vector-based shRNA<sup>[2,3]</sup>. A few promoters have been used to drive shRNA expression in cells, including the U6 and H1 RNA polymerase III promoters, and the cytomegalovirus (CMV) RNA polymerase II promoter.

However, the use of these promoters to drive shRNA expression *in vivo* would silence a given gene in all cell types, and thus produce undesirable effects in non-target cells. Cell-specific targeting of siRNA is an important issue to consider in RNAi therapy<sup>[4]</sup>. Before RNAi can be used to treat metastatic cancers, however, good cellular targets must be found<sup>[5]</sup>. To silence a gene specifically in tumor cells, without affecting its expression in normal cells, a tumor-specific promoter has to be used. To date, there are no reports of a tumor-specific promoter to drive shRNA expression. Recently, cell-specific gene silencing has been

achieved by using cell-specific promoters (SP-C) to drive shRNA expression, and a loxP-CRE regulatable RNAi system has also been developed<sup>[6,7]</sup>.

In the present study, we attempted to develop a tumor-specific RNAi system directly under the control of the hTERT promoter. We investigated whether this system could depress the expression of exogenous reporter genes, firefly luciferase (*LUC*) and enhanced fluorescent protein (*EGFP*) and an endogenous gene (*Bcl-2*). We constructed corresponding vectors containing various shRNAs driven by hTERT promoters. Using these vectors, we demonstrated efficient silencing of target genes specific to tumor cells, but not in normal cell *in vitro*. We also established HeLa cells stably expressing shRNA to target *Bcl-2*, which could increase the chemosensitivity of cells to 5-fluorouracil.

## Materials and methods

**Plasmid construction** To construct an hTERT promoter-driven shRNA vector (pBlock-TRTP-shRNA-mpA), the hTERT promoter, including 413 bp upstream from the transcription initiation site, was cloned from pGL3B-TRTP (a kind gift from Dr Mary CUSTER, National Cancer Institute, Bethesda, MD, USA) by polymerase chain reaction (PCR) and inserted into pSilencer4.1 (Ambion, USA) to replace the CMV promoter. A minimal poly(A) sequence (5'-TTATTTCC TAGAAAATAAAAGTAACCTAGACACACAACCAAA AAACATACGCCGCGA-3') was used as a terminal sequence. The DNA oligonucleotides coding for the shRNA were inserted between *Bam*HI and *Hind*III restriction sites. shRNAs targeting *LUC*, *EGFP*, *Bcl-2*, and an unrelated shRNA negative control (control) were synthesized: shLUC, sense 5'-GATCCCTTACGCTGAGTACTTCGATTCAAGAGATCGAAGTACTCAGCGTAAGTTA-3', antisense 5'-AGCTTAACTTACGCTGAGTACTTCGATCTCTTGAATCGAA-TACTCAGCGTAAG-3'; shEGFP, sense 5'-GATCCCAAA-GCTGGAGTACAACACTTCAAGAGAGTAGTTGTACTCCA-GCTTGTGTTA-3', antisense 5'-AGCTTAACACAAGCTGGAGTACAACACTTCTCTTGAAGTAGTTGTACTCCA-GCTTGTGG-3'; shBcl-2, sense 5'-GATCCGTACATCCATTATAAGCTGTTTCAAGAGAACAGCTTATAATGGATGTACTT-3', antisense 5'-AGCTTAAGTACATCCATTATAAGCTGTTCTCTTGAACAGCTTATAATGGATGTACG-3'; shControl, sense 5'-ATCCACTACCGTTGTTATAGGTGTTCAAGAGACACTATAACAACGGTAGTA-3', antisense 5'-AGCTTACTACCGTTGTTATAGGTGTCTCTTGAACA-CCTATAACAACGGTAGTG-3'. The target sequences were selected based on published synthetic siRNA sequences<sup>[6,8,9]</sup>. To facilitate the analysis of shRNA efficacy, an sv40-driven

luciferase-expressing cassette from pGL3-promoter was separately inserted into pBlock-TRTP-shLUC-mpA and pBlock-TRTP-shControl-mpA between *Sal*I and *Not*I restriction sites. The CMV-driven EGFP-expressing cassette from pGL3-CMV-EGFP (constructed with a framework of pGL3-promoter, with the CMV promoter from pCDNA3.0 replacing the sv40 promoter between *Bgl*II and *Hind*III restriction sites, and the *EGFP* gene from pEGFP-N1 replacing the luciferase gene between *Hind*III and *Xba*I restriction sites) was separately inserted into pBlock-TRTP-shEGFP-mpA and pBlock-TRTP-shControl-mpA between *Sal*I and *Not*I restriction sites. The method used was similar to the one described by Zeng *et al*<sup>[10]</sup>.

**Cell culture and transfection** HeLa, HepG2, and A549 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum (BCS), 100 U/mL penicillin and 100 mg/mL streptomycin. WI-38 cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin. The cells were transfected in opti-MEM with the transfection reagent Lipofectamine-2000 (Invitrogen, USA) according to the manufacturer's instructions. To establish HeLa cells stably expressing shRNA, cells were treated with 2 µg/mL puromycin (Sigma, USA) for 14 d, and after that cells continued to be cultured with 1 µg/mL puromycin.

**Luciferase assay** HeLa, HepG2, A549 and WI-38 cells were transfected in 24-well culture plates with 0.8 µg firefly luciferase (Fr-luc)-expressing vectors (pBlock-TRTP-shLUC-mpA-sv40-luciferase or pBlock-TRTP-shControl-mpA-sv40-luciferase) and 0.01 µg Renilla luciferase (Re-luc)-expressing vector (pRL-sv40) as transfection controls. The cells were harvested 48 h after transfection. The luciferase activity was measured with a dual luciferase assay kit (Promega, USA) using a luminometer (Lumat LB 9506, Berthold Technology). The relative luciferase activity was defined as the ratio of Fr-luc activity to Re-luc activity.

**Reverse transcription polymerase chain reaction analysis** After being treated with DNase, 2 µg of total RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen, USA) in the presence of random primers. The cDNA was used to amplify the *EGFP* or *Bcl-2* fragments. For normalization of RNA loading, the housekeeping gene  $\beta$ -actin was also amplified from each sample. The primer sequences were as follows: *EGFP*, sense 5'-TGCCACCTACGGCAAGCTGA-3', antisense 5'-TCGATGTTGTGGCGGATCTT-3'; *Bcl-2*, sense 5'-CGACGACTTCTCCCCTACCGCTACCGC-3', antisense 5'-CCGCATGCTGGGGCCGTACAGTTCC-3';  $\beta$ -actin, sense 5'-CCAAGGCCAACCGCGAGAAGATGAC-3', antisense 5'-AGGGTA-

CATGGTGGTGCCGCCAGAC-3'. PCR amplification was performed using the following conditions: 1 cycle of 95 °C for 5 min; for *EGFP* 25 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s; for *Bcl-2*, 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s; followed by a final elongation step of 72 °C for 10 min. After amplification, 10 µL of PCR product was run on a 1.5% agarose gel and visualized by ethidium bromide staining.

**Fluorescence-activated cell sorting analysis** HepG2, HeLa, A549, and WI-38 cells were transfected in 6-well culture plates with pBlock-TRTP-shEGFP-mpA-CMV-EGFP or pBlock-TRTP-shControl-mpA-CMV-EGFP. Forty-eight hours after transfection, the cells were photographed by using a fluorescence microscope and then harvested. Half of the cells were used to extract total RNA for reverse transcription (RT)-PCR, the others to observe the levels of *EGFP* fluorescence using fluorescence-activated cell sorting (FACS; calibur, BD, USA).

The cell cycle distribution profile was analyzed by FACS. A total of  $1 \times 10^6$  cells were collected and washed with phosphate-buffered saline (PBS), fixed in ice-cold 70% ethanol and stored at 4 °C. After being resuspended in PBS containing 100 µg/mL RNase A and 20 µg/mL propidium iodide (PI) for 30 min, samples were analyzed by FACS.

**MTT assay** The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was performed to assess the effect of *Bcl-2* on cell proliferation and chemosensitivity to 5-fluorouracil. Cells were plated in 96-well plates at a density of  $5 \times 10^3$  cells per well. Then for 7 d, every 24 h a batch of cells were stained with 20 µL sterile MTT dye (5 mg/mL; Sigma, USA) at 37 °C for 4 h, then culture medium was removed and 150 µL of Me<sub>2</sub>SO was added and thoroughly mixed in for 10 min. Spectrometric absorbance at 570 nm was measured by using a microplate reader. Each group contained 4 wells. To assess chemosensitivity to 5-fluorouracil, cells cultured for 24 h were incubated with different concentrations of 5-fluorouracil for another 48 h. Then cells were treated with MTT as described earlier. The cell survival value index was calculated as  $[A_{570(5\text{-fluorouracil}^+)} / A_{570(5\text{-fluorouracil}^-)}] \times 100\%$ .

**Western blotting** Cells were lysed in RIPA buffer [1% NP-40, 0.1% sodium dodecylsulfate (SDS), 0.5% deoxycholic acid (DOC), 150 mmol/L NaCl, 10 mmol/L Tris-HCl, and phenylmethylsulfonyl fluoride (PMSF)] at 4 °C for 30 min. An equal volume of lysate was electrophoresed with SDS-polyacrylamide gel electrophoresis (PAGE; 12%). The separated proteins in the gel were transferred to the nitrocellulose membrane. The membranes were blocked with Tris-buffered saline plus 0.1% Tween-20 (TTBS) containing 5%

non-fat milk for 1 h, incubated with the appropriate primary antibodies [anti *Bcl-2* (Santa Cruz, USA); anti-β-actin (Sigma)] in TTBS containing 5% non-fat milk for 2 h, followed by incubation with secondary antibodies for 1 h. Finally, the proteins were visualized by enhanced chemiluminescence reagents (Pierce, USA) and the specific bands were recorded on X-ray film.

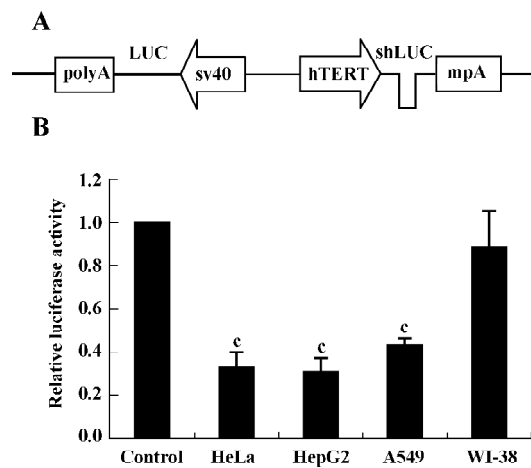
**Statistical analysis** All experiments were performed in triplicate. Student's *t*-test was used to determine the statistical significance of the data obtained.  $P < 0.05$  was taken to represent a statistically significant difference between means.

## Results

**Construction of shRNA-expressing vectors with hTERT promoter** We constructed an hTERT promoter-driven shRNA-expressing vector (pBlock-TRTP-shRNA-mpA). The shRNAs targeting *LUC*, *EGFP*, *Bcl-2* and negative control were separately cloned into pBlock-TRTP-shRNA-mpA, and the corresponding plasmids were named pBlock-TRTP-shLUC-mpA, pBlock-TRTP-shEGFP-mpA, pBlock-TRTP-shBcl-2-mpA and pBlock-TRTP-shControl-mpA, respectively. All constructs were sequence-verified.

**Effect of shRNA on exogenous reporter expression** We first tested whether hTERT promoter-driven shLUC could inhibit the expression of firefly luciferase. To facilitate the analysis of shRNA efficacy, we constructed the pBlock-TRTP-shLUC-mpA-SV40-luciferase (pBshLUC, Figure 1A) and pBlock-TRTP-shControl-mpA-SV40-luciferase (pBLUC) vectors. Cells were transfected with the Fr-luc-expressing vectors (pBshLUC or pBLUC) and the Re-luc-expressing vector pRL-sv40. Luciferase activity was determined by a dual luciferase assay 48 h after transfection. The relative luciferase activity was defined as the ratio of Fr-luc activity to Re-luc activity. The mean pBshLUC/pBLUC ratios were  $0.327 \pm 0.070$ ,  $0.308 \pm 0.063$  and  $0.426 \pm 0.034$  in HeLa, HepG2 and A549 tumor cells, respectively ( $P < 0.01$  vs control; Figure 1B), whereas the mean pBshLUC/pBLUC ratio was  $0.880 \pm 0.172$  in WI-38 cells.

Next we investigated the inhibitory effects of hTERT promoter-driven shRNA targeted to another exogenous reporter, *EGFP*. We also constructed pBlock-TRTP-shEGFP-mpA-cmv-EGFP (pBshEGFP; Figure 2A) and pBlock-TRTP-shControl-mpA-cmv-EGFP (pBEGFP) vectors. The concentration of EGFP transcripts was determined by RT-PCR, normalized to the concentration of β-actin mRNA. We found that the concentration of *EGFP* transcripts decreased in HepG2, HeLa and A549 cells transfected with pBshEGFP, relative to controls, whereas there was no change in WI-38



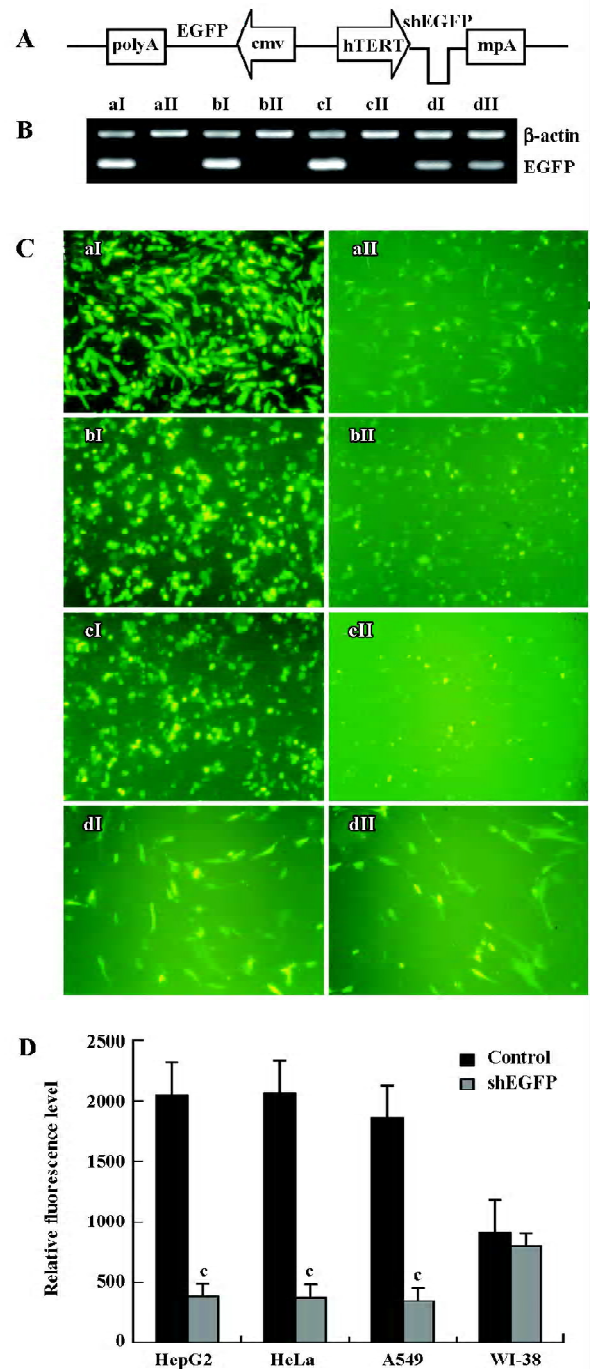
**Figure 1.** Inhibition of luciferase expression by shLUC. (A) Schematic diagram of pBlock-hTRTP-shLUC-mpA-SV40-luciferase. (B) shLUC efficacy in various cells. HeLa, HepG2, A549 and WI-38 cells were transfected with pBshLUC or pBLUC (Fr-luc), and the Renilla luciferase (Re-luc)-expressing vector pRL-sv40 served as a transfection control. The relative luciferase activity was defined as the ratio of Fr-luc activity to Re-luc activity. The activity of pBLUC·Re-luc (control) was designated as 1. Each group contained 3 wells and experiments were repeated 3 times. \* $P < 0.01$  vs control.

normal cells (Figure 2B).

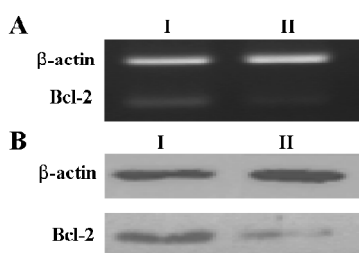
The inhibitory effects of shEGFP on EGFP protein were evaluated by fluorescence microscopy and FACS. The relative level of EGFP fluorescence did not change in WI-38 cells, but dropped markedly in HepG2, HeLa and A549 cells transfected with pBshEGFP, as determined by direct visualization under a fluorescence microscope (Figure 2C) and FACS analysis ( $P < 0.01$ ; Figure 2D). The results were consistent with those at the mRNA level.

**Effect of shRNA on Bcl-2 expression** We next tested whether the shRNA controlled by the hTERT promoter worked on the endogenous gene *Bcl-2*. HeLa cells were transfected with pBlock-TRTP-shBcl-2-mpA (pBshBcl-2) or pBlock-TRTP-shControl-mpA (pBControl). For establishing HeLa cells stably expressing shRNA, cells were treated with 2  $\mu\text{g/mL}$  puromycin for 14 d, and after that cells continued to be cultured with 1  $\mu\text{g/mL}$  puromycin. The level of *Bcl-2* expression did not change in HeLa cells stably transfected with pBControl relative to that in untransfected cells (data not shown), but was markedly reduced in cells stably transfected with pBshBcl-2, as evaluated by RT-PCR and Western blotting (Figure 3A, 3B).

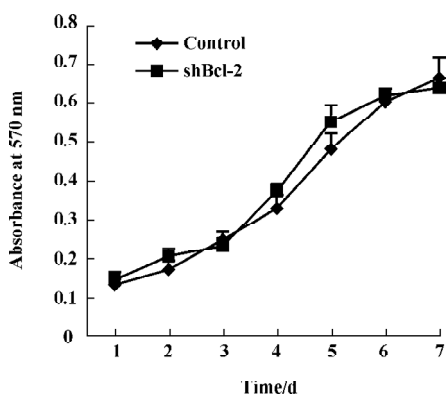
**Effect of Bcl-2 expression on proliferation and cell cycle** The proliferation rates of HeLa cells stably expressing shBcl-2 and shControl were similar, as determined by MTT chromometry (Figure 4). We also used FACS to analyze the cell



**Figure 2.** Inhibition of EGFP expression by shEGFP. (A) Schematic diagram of pBlock-TRTP-shEGFP-mpA-cmv-EGFP. (B) EGFP expression levels as measured by RT-PCR. HepG2, HeLa, A549 and WI-38 cells were transfected with pBshEGFP or pBEGFP. Lanes: a, HepG2; b, HeLa; c, A549; d, WI-38; I, cells transfected with pBEGFP; II, cells transfected with pBshEGFP. (C) EGFP expression levels as assessed by fluorescence microscopy ( $\times 100$ ). Cells were photographed 48 h after transfection with pBEGFP or pBshEGFP. The part labels are the same as those in part (B). Scale bar, 20  $\mu\text{m}$ . (D) Relative EGFP fluorescence levels as assessed by FACS.  $n = 3$ . Mean  $\pm$  SD. \* $P < 0.01$  vs control.



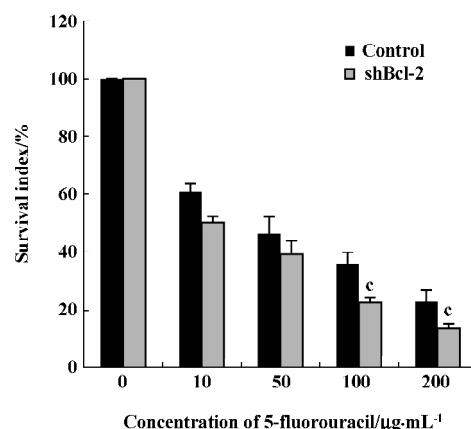
**Figure 3.** hTERT promoter-directed synthesis of shRNA silenced endogenous *Bcl-2* expression. (A) *Bcl-2* expression level as assessed by RT-PCR. HeLa cells were transfected with pBlock-TRTP-sh*Bcl-2*-mpA (pBsh*Bcl-2*) or pBlock-TRTP-shControl-mpA (pBshControl). Cells stably expressing shRNA were selected using puromycin. Lanes: I, transfected with pBshControl; II, transfected with pBsh*Bcl-2*. (B) The level of *Bcl-2* protein as assessed by Western blotting. The lane numbers have the same definition as those in A.



**Figure 4.** Proliferation of HeLa cells stably expressing shRNA. HeLa cells stably transfected with pBlock-TRTP-sh*Bcl-2*-mpA or pBlock-TRTP-shControl-mpA were plated in 96-well plates at a density of  $5 \times 10^3$  cells per well. Every 24 h, for 7 d, a batch of cells were stained with 20  $\mu$ L sterile MTT dye (5 mg/mL) at 37 °C for 4 h, then culture medium was removed and 150  $\mu$ L of  $\text{Me}_2\text{SO}$  was added and thoroughly mixed in for 10 min. Spectrometric absorbance at 570 nm was measured using a microplate reader. Each group contained 4 wells and the experiments were repeated 3 times.

cycle distribution profile. Inhibition of *Bcl-2* did not influence the cell cycle profile (data not shown), suggesting that the *Bcl-2* gene did not significantly affect cell growth or viability.

**Effect of *Bcl-2* expression on chemosensitivity to 5-fluorouracil** Because the *Bcl-2* gene has been found to play an important role in resistance to chemotherapy in many tumors, we next investigated if inhibition of *Bcl-2* by RNAi affected the sensitivity of HeLa cells to the anti-tumor drug 5-fluorouracil. The survival index in HeLa cells stably expressing sh*Bcl-2* was markedly reduced with the addition of 100  $\mu$ g/mL or 200  $\mu$ g/mL 5-fluorouracil ( $0.225 \pm 0.020$  vs  $0.359 \pm$



**Figure 5.** Effect of different concentrations of 5-fluorouracil on HeLa cells. HeLa cells stably expressing sh*Bcl-2* and shControl were treated with various concentrations of 5-fluorouracil. Cell viability was determined by MTT chromometry. Cell survival index was calculated as  $[A_{570(5\text{-Fluorouracil}+)} / A_{570(5\text{-Fluorouracil}-)}] \times 100\%$ .  $^{\circ}P < 0.05$  vs control.

$0.039, 0.125 \pm 0.019$  vs  $0.228 \pm 0.040$ ;  $P < 0.05$ , Figure 5) relative to controls.

## Discussion

Utilization of a promoter that is predominantly active in tumor cells would be an ideal strategy for restricting therapeutic gene expression. Telomerase is a particularly attractive target because approximately 90% of tumor cells have telomerase activity, whereas most normal cells do not express that enzyme. In human cells, telomerase is under the transcriptional control of the hTERT gene, whose product is the catalytic subunit of the enzyme<sup>[16]</sup>. Transcriptional activity of the hTERT promoter is highly specific to telomerase-positive tumor cells. Therefore, the hTERT promoter has been widely used in targeting cancer gene therapy to restrict the expression of exogenous genes to various cancer cells<sup>[12-14]</sup>. A recent report also showed that the hTERT promoter might be a good, safe candidate for gene therapy<sup>[15]</sup>. To determine whether the hTERT promoter can be used to produce shRNA and silence gene expression in a tumor-specific fashion, we constructed a vector in which shRNA in the form of a small hairpin structure was placed under the control of the 403 bp hTERT promoter followed by a minimal polyA (mpA) sequence. A similar mpA has been used successfully for CMV and SP-C promoter-driven shRNA<sup>[6,11]</sup>.

In the present study, we demonstrated that hTERT promoter-driven shRNA could depress the expression of the exogenous reporter genes (firefly luciferase and *EGFP*) in different tumor cells (HeLa, HepG2 and A549) but not normal

cells (WI-38). WI-38 cells are human fetal lung fibroblasts, which have no endogenous hTERT expression<sup>[16]</sup>, and are often used as representative normal cells<sup>[17,18]</sup>. hTERT promoter-driven shLUC decreased luciferase expression by approximately 70% according to the dual luciferase assay. The efficacy was similar to that of chemically synthesized siRNA or other DNA vector-based shRNA targeted to luciferase<sup>[8]</sup>. shEGFP driven by the hTERT promoter depressed the mRNA and protein expression of *EGFP* to the same level. This reveals that shRNA synthesized by the hTERT promoter silences gene expression in a sequence-specific manner through targeting mRNA for endonucleolytic cleavage.

We next tested whether the hTERT promoter-driven shRNA worked on the endogenous gene *Bcl-2*. *Bcl-2* is an apoptotic inhibitor that may contribute to therapeutic resistance. It is the first oncogene found to function through production of an inhibitor of programmed cell death<sup>[19]</sup>. Overexpression of *Bcl-2* is common in many human cancers, and contributes to increased resistance to chemotherapy<sup>[20-22]</sup>. *Bcl-2* antisense oligonucleotides can increase the anticancer drug sensitivity of tumor cells<sup>[23,24]</sup>. Recently, successful attempts were made to silence *Bcl-2* using RNAi techniques<sup>[9,25]</sup>. RNAi techniques have been repeatedly proven to be more robust than antisense techniques because they work more often, typically decrease expression of a gene to lower level, and also act at concentrations below those required for antisense techniques<sup>[25]</sup>. Therefore, antisense strategies may be replaced by RNAi approaches. Our study showed that sh*Bcl-2* driven by the hTERT promoter could depress the expression of *Bcl-2* with respect to both mRNA and protein levels. Suppression of *Bcl-2* did not influence cell growth, but increased chemosensitivity to 5-fluorouracil in HeLa cells. Our results regarding the significant biological effects of tumor-specific RNAi against the *Bcl-2* gene might be considered promising for future RNAi-based therapeutic approaches in HeLa cells or other malignant cell types. Using this strategy, some oncogenes could also be depressed in tumor cells without interfering with other cell systems.

In conclusion, we report here the first tumor-specific RNAi system using the hTERT promoter. We demonstrated that hTERT promoter-driven shRNA expression could effectively and specifically silence gene expression in different tumor cells but not in normal cells *in vitro*. We also showed that inhibition of *Bcl-2* expression with this system could increase chemosensitivity to 5-fluorouracil in HeLa cells. This system might be a good candidate for future RNAi therapy.

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## American Society for Clinical Pharmacology and Therapeutics 2006 Annual Meeting

2006 March 8–11

Baltimore Marriott Waterfront  
Baltimore, MD, USA  
<http://www.ascpt.org>