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# *hTERT* and *BIRC5* gene promoters for cancer gene therapy: A comparative study

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**Abstract.** Human telomerase reverse transcriptase (*hTERT*) and survivin (*BIRC5*) gene promoters are frequently used for transcriptional targeting of tumor cells, yet there is no comprehensive comparative analysis allowing rational choice of a promoter for a particular therapy. In the current study, the transcriptional activity of *hTERT*, human *BIRC5* and mouse *Birc5* promoters and their modifications were compared in 10 human cancer cell lines using the luciferase reporter gene activity assay. The results revealed that *BIRC5*- and *hTERT*-based promoters had strikingly different cell specificities with comparable activities in only 40% of cell lines. Importantly, relative *hTERT* and *BIRC5* transcript abundance cannot be used to predict the most potent promoter. Among the *hTERT*-based promoters that were assessed, modification with the minimal cytomegalovirus promoter generally resulted in the most potent activity. Mouse *Birc5* and modified human *BIRC5* promoters were superior to the unmodified human survivin promoter; however, their tumor specificities must be investigated further. In summary, the present results emphasize the desirability for construction of more universal tumor-specific promoters to efficiently target a wide spectrum of tumor cells.

## Introduction

Transcriptional targeting in gene therapy is an approach based on the use of tissue-specific or tumor-specific promoters (TSPs) to direct the expression of therapeutic genes specifically to a tumor (1). It is assumed that it must meet the requirements of transgene expression in tumor tissues but not in normal tissues. This combination of 'tumor on' and 'normal tissue off' profile can result in an increase in the therapeutic index and limit the toxicity of vectors and transgenes *in vivo*. Numerous promoters have already been evaluated for transcriptional targeting in cancer gene therapy [for reviews, see (2-4)], such as the α-fetoprotein promoter for hepatic carcinoma, tyrosinase gene promoter for melanoma, prostate-specific antigen promoter for prostate cancer, cyclooxygenase-2 promoter for gastrointestinal cancer, midkine promoter for Wilms' tumor or neuroblastoma, chemokine (C-X-C motif) receptor 4 promoter for breast cancer and melanoma, hypoxia-inducible promoter for hypoxic tumor-targeting gene therapy, promoter of the carcinoembryonic antigen gene (5), and numerous others. However, many of the gene promoters used for this goal have been shown to be abundantly expressed in a variety of normal tissues (4), such that their usage also affects normal host cells, thus increasing the risk of unwanted side effects. TSPs that are expressed in a wide variety of tumors and have low expression in normal tissue are highly desirable to meet gene therapy demands. Two gene promoters frequently used for the gene therapeutic purposes appear to most completely satisfy the requirements: The human telomerase reverse transcriptase (*hTERT*) promoter, and the promoter driving the expression of *BIRC5*, encoding the apoptosis inhibitor survivin.

Survivin is one of the central players of cancer development (1,6-8), and the *BIRC5* gene (9) promoter is also active in wide spectrum of cancer cells (10-13). In normal tissues, survivin expression is found during embryonic and fetal development but is largely undetectable in terminally differentiated adult tissues (14). Although growing evidence indicates that survivin is expressed in primitive hematopoietic cells, T lymphocytes, polymorphonuclear neutrophils and vascular endothelial cells, and that it may regulate their proliferation or survival, targeted anti-survivin therapies have exhibited efficacy without overt toxicity in numerous preclinical animal models (14). It has been reported that the functional promoter region of the *BIRC5* gene spans 1,456 bp upstream of the transcription start site (15) and continues to ~40 nucleotides (nt) downstream of the transcription start point. The *BIRC5*

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*Abbreviations:* TSP, tumor-specific promoter; hTERT, human telomerase reverse transcriptase

*Key words:* hTERT promoter, survivin/BIRC5 promoter, comparative study, tumor-specific transcription, cancer gene therapy

gene promoter is highly tumor-specific and works in a great majority (80-85%) of tumors (9,14,16), thus presenting the possibility of its general utilization in cancer treatment (17). However, in common with the majority of TSPs (17-19), it is rather weak in comparison with such promoters as constitutive cytomegalovirus (CMV) and Simian vacuolating virus 40 (SV40) promoters with their enhancers (when used as isolated promoters in the context of a vector to drive transgene expression). *BIRC5* promoter activity in different cell lines comprises 0.3-16% of that of a strong constitutive CMV promoter (4,13,18,20). Therefore, it is highly desirable to reconstruct the promoter so that it acquires a higher activity level while remaining strictly cancer-specific.

Recently, we constructed a modified version of the human *BIRC5* promoter ($P_{hSurv}$) by inserting fragment D (326 nt), consisting of the first exon and a part of the first intron of the human *BIRC5* gene, which contains a CpG-island and possesses enhancer-like activity (Fig. 1A). This modified promoter, referred to as $P_{hSurvD}$, exhibited enhanced transcriptional activity in the majority of p53-negative lung cancer cell lines (21). It must be noted that shortening of the human *BIRC5* promoter fragment beyond 1.4 kb upstream of the transcription start site resulted in decreased promoter activity (15). At the same time, the overall expression cassette length could be a concern for gene therapy application due to the limited capacity of certain vectors used for delivery, such as that of adenoviral vectors (22). Therefore, short promoters that are able to retain tumor specificity and possess maximal activity are preferred. A short fragment of the murine *Birc5* gene promoter spanning nt -155 to +42 relative to the transcription start site [corresponding to nt -195 to +2 as numbered in (23,24)] (Fig. 1B) was reported to be highly active in an *in vitro* reporter gene analysis (23) and demonstrated higher transgene expression in murine cells than that observed in human cells with the human *BIRC5* promoter (20), thus providing another option for improved survivin promoter-driven expression in tumor cells.

Telomerase activity is critical for the acquisition of immortality by cancer cells through maintaining telomere length. In humans, telomerase activation in cancer cells is achieved due to restoring the telomerase reverse transcriptase subunit of telomerase; this relies on transcriptional activation of the *hTERT* gene, which is silent in somatic human tissues. *hTERT* expression has been observed in various types of human cancer (25-27), with human telomerase being highly active in >85% of primary cancers, regardless of their tissue origins, but not in normal differentiated human cells (26,28-31). It has been revealed and later confirmed in numerous studies that a ~200-bp fragment of the *hTERT* 5'-flanking region is enough to function as a promoter for transcriptional activation in cancer cells while maintaining cancer cell specificity (29). The *hTERT* promoter lacks TATA- and CAAT-boxes (29,32,33). In an attempt to increase the promoter activity critical for improved transgene expression, the promoter was modified to increase its activity without appreciable loss of cancer cell specificity. One of the reported modifications consisted of the joining of $P_{hTERT}$ with a minimal CMV promoter (Fig. 1C) (34). In another modification, a TATA-box derived from the adenoviral E1A promoter was added to *hTERT* promoter (Fig. 1C) (35). These two modifications were reported to improve the promoter performance without compromising its cancer cell specificity. However, no direct comparison of these two modifications has been previously conducted to identify which one is preferable to drive transgene expression in cancer cells.

Therefore, the *BIRC5* and *hTERT* promoters and their modified versions may be good candidates for use as a TSPs in gene therapy approaches to treat cancers, with a low potential toxicity for normal host tissue *in vivo*. However, despite the widespread use of the two promoters in experimental studies of potential gene therapy schemes, to the best of our knowledge there is no comprehensive comparative analysis that would allow a comparison of the advantages and disadvantages of each promoter and determine a rational basis for selecting the optimal promoter for gene therapy of a particular tumor. Such a comparison would also be of use in the creation of more universal synthetic promoters bearing combinations of elements from these two promoters. In the present study, such a comparison was conducted, revealing that activities of *hTERT*- and *BIRC5*-based promoters are, in many cases, complementary to one another, such that if one of them fails to support efficient therapeutic gene expression in a tumor, the other may be used instead.

## Materials and methods

*Cell lines*. Human HT1080 fibrosarcoma (#CCL-121; ATCC, Manassas, VA, USA), Calu-I epidermoid carcinoma of lung (#93120818; ECACC, Porton Down, UK), NCI-H1299 non-small cell lung carcinoma (#CRL-5803; ATCC), AsPC-1 adenocarcinoma of the pancreas (#CRL-1682; ATCC), Panc-1 ductal carcinoma of the pancreas (#CRL-1469; ATCC) and HCT116 colorectal carcinoma (#CCL-247; ATCC) cell lines were cultured in HyClone Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1) (GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone; GE Healthcare Life Sciences), penicillin (100 U/ml) and streptomycin (100 $\mu$g/ml). T47D breast ductal carcinoma (#HTB-133; ATCC) and A431 epidermal squamous carcinoma (#CRL-1555; ATCC) cells were cultured in HyClone DMEM supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 $\mu$g/ml). The human melanoma cell lines MelCher and MelKor (36) were cultured in HyClone RPMI-1640 medium (GE Healthcare Life Sciences) supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 $\mu$g/ml). Cells were transfected using Unifectin-56 transfection reagent (Rusbiolink, Moscow, Russia) (HCT116, HT1080, Panc-1, Calu-I, NCI-H1299 and MelKor cell lines), or Lipofectamine 2000 (Thermo Fisher Scientific, Inc., Eugene, OR, USA) (AsPC1, MelCher, A431 and T47D cell lines).

*Plasmids*. The human *BIRC5* promoter fragment nt -1,456 to +42 ($P_{hSurv}$; nt 76,208,870 to 76,210,367; GenBank accession no. NC_000017.10) was amplified on a template of human genomic DNA with primers 5'-agatctaaatctgggtgaaggtatatgagt-3' and 5'-aagcttcgcgattcaaatctggcggt-3' and cloned into the pGL3-Basic vector (Promega Corporation, Madison, WI, USA) to control firefly luciferase reporter gene expression (Fig. 1A).

Fragment D (nt +43 to +368 of the human *BIRC5* gene) was amplified on a template of human genomic DNA with primers 5'-cccgggacccgttggcagaggtggcggcggcggc(a>t)tggg-3' [with the

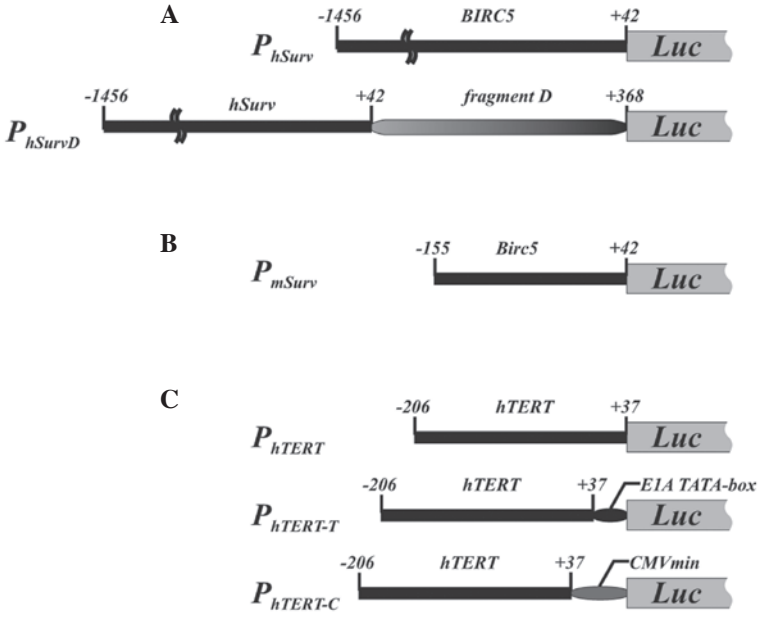


Figure 1. Schematic representation of the firefly luciferase reporter constructs. (A) Human survivin promoter series of the reporter plasmids with $P_{hSurv}$ promoter fragment spanning nt -1456 to +42 of the human *BIRC5* gene, and its modification with fragment D, $P_{hSurvD}$. (B) Mouse survivin promoter $P_{mSurv}$ plasmid containing nt -155 to +42 of the mouse *Birc5* gene. (C) hTERT promoter series of the reporter plasmids with $P_{hTERT}$ encompassing nt -206 to +37 of the *hTERT* gene, and its modifications on the 3'-end with adenoviral E1A TATA-box ($P_{hTERT-T}$), and a minimal cytomegalovirus (*CMVmin*) promoter ($P_{hTERT-C}$). nt, nucleotides; BIRC5, survivin; hTERT, human telomerase reverse transcriptase.

A>T substitution (indicated) in order to replace the ATG translation initiation codon of the *BIRC5* gene by a TTG triplet] and 5'-taagcttcctcgatggggacaaagcag-3', and cloned at the 3'-end of $P_{hSurv}$ (promoter $P_{hSurvD}$). The orientation of fragment D in the $P_{hSurvD}$ coincided with that in human genomic DNA (Fig. 1A).

The mouse *Birc5* gene promoter fragment nt -155 to +42 ($P_{mSurv}$; nt 117,710,470 to 117,710,664; GenBank accession no. NC_000077.5) was amplified on the template of mouse genomic DNA with primers 5'-agatctccacgcccacaaggccag gc-3' and 5'-aagcttatgatggcgtcaccacaacc-3', and cloned into the pGL3-Basic vector (Fig. 1B).

The *hTERT* promoter fragment nt -206 to +37 ($P_{hTERT}$; nt 110,006 to 11,248; GenBank accession no. AF128893) was amplified on the template of human peripheral blood lymphocyte genomic DNA and cloned into pGL3-Basic vector to place the firefly luciferase reporter gene under the control of $P_{hTERT}$ (Fig. 1C). To construct a hybrid $P_{hTERT}$ promoter with a synthetic TATA-box ($P_{hTERT-T}$ promoter), complementary oligonucleotides with an adenoviral E1A TATA-box sequence (5'-aattcgtgtagtgtatttatacccggtgagtagatctg-3' and 5'-gatccagat ctactcaccgggtataaatacactacacg-3') (35) were annealed and linked to the 3'-end of $P_{hTERT}$. The resulting $P_{hTERT-T}$ fragment was cloned into pGL3-Basic vector to drive firefly luciferase reporter gene expression (Fig. 1C). Hybrid telomerase reverse transcriptase promoter ($P_{hTERT-C}$) with a minimal CMV promoter derived from the pTRE-Tight plasmid (Clontech Laboratories, Inc., Mountain View, CA, USA) was constructed in a similar way with the 69-nt minimal major immediate early CMV promoter fragment amplified with primers 5'-atgaattcg gtaggcgtgtacggtgggag-3' and 5'-atggatccagatctccaggcgatctga cgg ttc-3' on the template of the pTRE-Tight vector (Fig. 1C).

*Luciferase reporter assay*. Cells were transfected with a mixture of firefly reporter plasmid and a pRL-CMV (Promega Corporation) plasmid (encoding a *Renilla* luciferase reporter gene under the control of CMV immediate early enhancer/promoter) in a ratio selected for each cell line to result in optimal signals for each reporter. Cells in three wells were transfected in parallel for each plasmid combination. Along with plasmids with firefly reporter gene under the control of the studied promoters, a pGL3-Control plasmid (Promega Corporation) with a firefly luciferase reporter gene under the control of the SV40 promoter and enhancer sequences, and a promoter-less pGL3-Basic plasmid (Promega Corporation) were used. Luciferase activities were quantified 2 days after transfection either with Dual-Glo™ Luciferase Assay System or with Dual-Luciferase® Reporter Assay System (both from Promega Corporation) depending on the cell line transfection efficiency. Firefly luciferase activity was normalized to *Renilla* luciferase activity and then the mean ± standard deviation (SD) values were calculated from the values for three analyzed wells for each experimental point. The data presented are the mean± SD following the subtraction of basal activity of the promoter-less reporter (plasmid pGL3-Basic). Values were considered statistically significant if the two-tailed P-value (unpaired *t*-test) was <0.05.

*Relative transcript level determination*. Total RNA was isolated from cells with RNeasy Mini RNA Kit (Qiagen GmbH, Hilden, Germany). Reverse transcription (RT) was performed on a template of 1 $\mu$g of RNA with random hexamer primers and SuperScript III reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Carlsbad, CA, USA). RT reaction products were diluted to give approximately equal amplification of *GAPDH* transcript across the samples. Polymerase chain reaction (PCR) was conducted in quadruplicates for each transcript analyzed in each cell line. The 20-$\mu$l reaction mixture contained 2 $\mu$l of 10X amplification buffer (100 mM

Table I. Promoter activities in various cell lines.

| Cell line | Promoter activity | | | | | |
|---|---|---|---|---|---|---|
| | $P_{hTERT}$ | $P_{hTERT-T}$ | $P_{hTERT-C}$ | $P_{hSurv}$ | $P_{hSurvD}$ | $P_{mSurv}$ |
| HCT116 | 1.00±0.17 | 2.63±0.48[a] | 8.01±0.82[a,b] | 0.48±0.26[a] | 1.34±0.23[c] | 1.56±0.19[c] |
| AsPC-1 | 1.00±0.54 | 1.32±0.15 | 4.40±0.49[a,b] | 0.33±0.04[a] | 1.16±0.11[c] | 2.19±0.11[c,d] |
| Panc-1 | 1.00±0.43 | 1.15±0.17 | 3.41±0.21[a,b] | 0.73±0.49 | 0.92±0.31 | 2.03±0.39[c,d] |
| HT1080 | 1.00±0.10 | 1.02±0.28 | 1.74±0.04[a,b] | 0.83±0.31 | 1.55±0.08[c] | 1.80±0.22[c] |
| Calu-I | 1.00±0.81 | 1.76±0.31 | 2.51±0.57[a] | 6.31±1.06[a] | 10.67±2.82 | 14.06±2.32[c] |
| NCI-H1299 | 1.00±0.16 | 1.15±0.09 | 1.97±0.07[a,b] | 2.40±0.24[a] | 3.34±0.07[c] | 3.60±0.26[c] |
| A431 | 1.00±0.22 | 1.68±0.34[a] | 1.33±0.14 | 8.56±2.75[a] | 20.34±9.08 | 17.66±4.78[c] |
| MelCher | 1.00±0.61 | 0.95±0.56 | 11.38±3.73[a,b] | 3.85±2.67 | 7.99±2.51 | 3.97±1.39 |
| MelKor | 1.00±0.07 | 2.20±0.06[a] | 1.47±0.04[a] | 0.17±0.01[a] | 0.73±0.08[c] | 1.17±0.10[c,d] |
| T47D | 1.00±0.08 | 0.74±0.04[a] | 1.42±0.06[a,b] | 0.72±0.09[a] | 2.24±0.18[c] | 2.79±0.13[c,d] |

Data are presented as mean ± standard deviation values of firefly luciferase reporter gene activities for indicated promoters after normalization to *Renilla* luciferase activity, referenced to $P_{hTERT}$ activity. [a]Significantly different (P<0.05) from the activity of $P_{hTERT}$ (for $P_{hTERT-T}$, $P_{hTERT-C}$ and $P_{hSurv}$). [b]Significantly different (P<0.05) from the activity of $P_{hTERT-T}$ (for $P_{hTERT-C}$). [c]Significantly different (P<0.05) from the activity of $P_{hSurv}$ (for $P_{hSurvD}$ and $P_{mSurv}$). [d]Significantly different (P<0.05) from the activity of $P_{hSurvD}$ (for $P_{mSurv}$). In some cases, data were replicated in independent experiments, with relative patterns of promoter strengths remaining essentially the same. hTERT, human telomerase reverse transcriptase; hSurv, human survivin; mSurv, mouse survivin.

Tris-HCl, pH 8.3, and 500 mM KCl), 0.8 $\mu$l of 25 mM magnesium chloride, 0.4 $\mu$l of 10 mM dNTP mix, 0.2 $\mu$l of each sense and antisense primer at 10 optical units/ml, 0.2 $\mu$l of Taq DNA polymerase (5 U/ml) and template. The primer pairs used were 5'-gaaggtgaaggtcggagtca-3' and 5'-ttcacacccatgacgaacat-3' for *GAPDH*; 5'-cggaagagtgtctggagcaa-3' and 5'-ggatgaagcggagtc tgga-3' for *hTERT*; and 5'-accgcatctctacattcaag-3' and 5'-gga ataaaccctggaagtgg-3' for *BIRC5*. Cycling conditions following initial heating at 94˚C for 2 min were 94˚C for 30 sec; 61˚C (*GAPDH*) or 63˚C (*hTERT*) or 60˚C (*BIRC5*) for 1 min; and 72˚C for 1 min. The number of cycles was 15, 18, 21 and 24 for *GAPDH*; 25, 29, 33 and 37 for *hTERT*; and 21, 25, 29 and 33 for *BIRC5*. Amplification products (402 bp for *GAPDH*, 145 bp for *hTERT* and 438 nt for major *BIRC5* transcript isoform) were resolved on an agarose gel containing ethidium bromide. An O'GeneRuler 50 bp DNA Ladder (Fermentas; Thermo Fisher Scientific, Inc.) was used to monitor amplification product lengths. Gel images were acquired with ChemiDoc XRS Documentation system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and band intensities were quantified with Quantity One 1-D Analysis Software (version 4.6.2; Bio-Rad Laboratories, Inc.). Relative *hTERT* and *BIRC5* transcript levels were determined using the band intensities at cycle numbers before reactions reached saturation by the amplification products (typically at 29 and 33 cycles for *BIRC5* and *hTERT*, respectively).

*Statistical analysis*. To determine significant differences between two values, an unpaired t-test was used to calculate two-tailed P-values using GraphPad QuickCalcs online tool (www.graphpad.com/quickcalcs/ttest1/). P<0.05 indicated a statistically significant difference. To evaluate correlation between two datasets, $r^2$ coefficient of correlation was calculated using the least squares method in Microsoft Excel 2011 for Mac (Microsoft Corporation, Redmond, WA, USA).

## Results

*Classification of cell lines according to the efficiency and pattern of various promoter activities*. $P_{hSurv}$ and $P_{hTERT}$ promoter activities were assessed in 10 cancer cell lines of various origins using firefly luciferase reporter gene and *Renilla* luciferase expressed under the control of a CMV promoter as a reference for normalization. For an appropriate comparison of activities of the promoters in different tumor cell lines, a proper reference control is necessary. We observed that the activity of the SV40 promoter and enhancer sequences, which are supposed to be constitutive, varied relative to the CMV promoter in a range of up to ~100 times for 5 cell lines investigated (data not shown). The observed difference indicates that SV40 promoter and enhancer sequences or/and CMV promoter activity cannot be used as a reference control. The possibility that the observed variations were due to the variability in activity of the CMV promoter driving *Renilla* luciferase expression used for firefly luciferase activity normalization could not be excluded. Thus an adequate comparative analysis was possible only for different promoters for a particular cell line, and not across different cells.

The studied tumor cell lines could be classified based on $P_{hTERT}$- or $P_{hSurv}$-driven promoter expression preference. According to this classification, there are $P_{hTERT}$ expressors (MelKor, HCT116, AsPC-1), $P_{hSurv}$ expressors (Calu-I, NCI-H1299, A431, MelCher), and cells with similar promoter activities (HT1080, Panc-1, T47D) (Table I). Among the cell lines analyzed, $P_{hTERT}$ activity relative to the activity of $P_{hSurv}$ ranged from 0.17 in A431 cells to 8.56 in MelKor cells, giving a 50-fold difference between $P_{hTERT}$ or $P_{hSurv}$ activities in different tumor cell lines. This observation clearly indicates the necessity for choosing between the two promoters for efficient transgene expression in a given tumor type.

Table II. Semi-quantified results of reverse transcription-polymerase chain reaction (Fig. 3).

| Transcript | NCI-H1299 | Calu-I | HT1080 | Panc-1 | HCT116 | AsPC-1 | MelCher | T47D | A431 | MelKor |
|---|---|---|---|---|---|---|---|---|---|---|
| hTERT | 0.92 | 0.14 | 0.54 | 1.00 | 0.59 | 0.73 | 0.31 | 0.16 | 0.75 | 0.49 |
| BIRC5 | 0.31 | 0.15 | 0.22 | 0.11 | 0.73 | 0.03 | 0.67 | 0.21 | 0.21 | 1.00 |
| Ratio | 0.34 | 1.10 | 0.40 | 0.11 | 1.24 | 0.04 | 2.17 | 1.38 | 0.29 | 2.04 |

Results indicate the relative levels of *hTERT* and *BIRC5* transcripts normalized to glyceraldehyde 3-phosphate dehydrogenase content, and the ratio of *BIRC5* to *hTERT* relative transcript levels, in the various cell lines. hTERT, human telomerase reverse transcriptase; BIRC5, survivin.

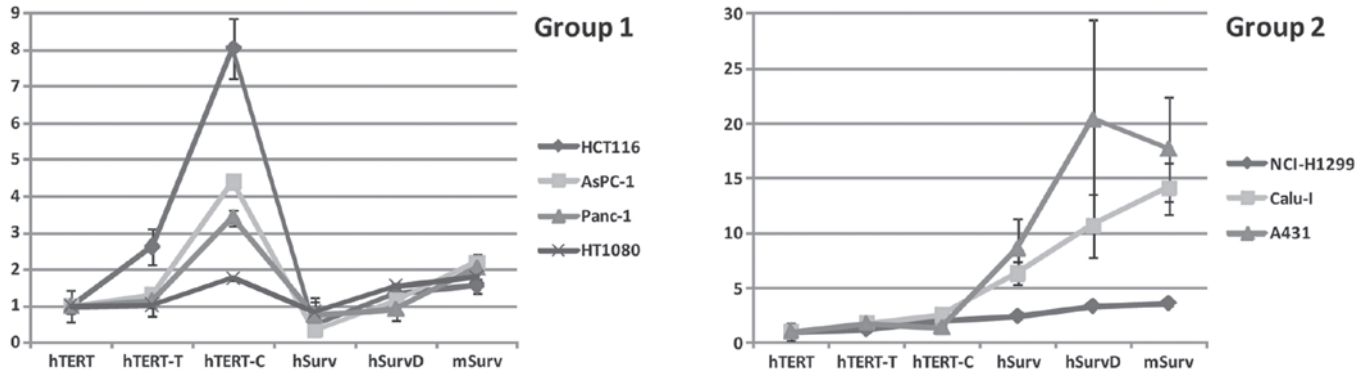


Figure 2. Clustering of cell lines into two groups (Group 1 and Group 2) on the basis of promoter expression patterns. Relative firefly luciferase activities under the control of the indicated promoters following normalization to *Renilla* luciferase activity and referencing to the activity of $P_{hTERT}$ (taken as 1.00) are shown as the mean ± standard deviation for indicated cell lines. hTERT, human telomerase reverse transcriptase; hSurv, human survivin; mSurv, mouse survivin.

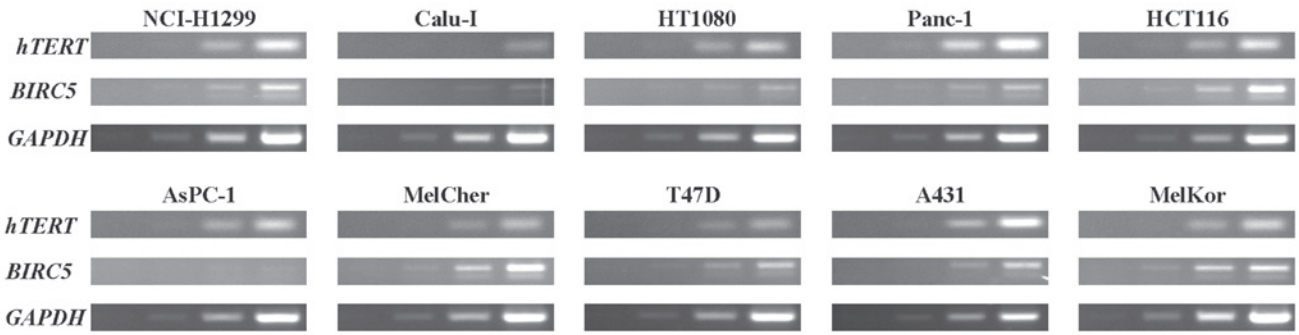


Figure 3. Results of reverse transcription-polymerase chain reaction analysis of *hTERT* and *BIRC5* transcript levels in indicated cell lines. Semi-quantification is presented in Table II. hTERT, human telomerase reverse transcriptase; BIRC5, survivin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

With regard to relative activities of various promoter variants used in the study, two different patterns can be distinguished. Panc-1, AsPC-1, HCT116 and HT1080 cell lines ('Group 1') had similar patterns of expression, with approximately the same (with 3-fold difference in range) activities of $P_{hTERT}$ or $P_{hSurv}$ which were significantly enhanced by modification of the $P_{hTERT}$ promoter (particularly $P_{hTERT-C}$) and when using other variants of the survivin promoter series, particularly $P_{mSurv}$ (Fig. 2). NCI-H1299, Calu-I and A431 ('Group 2') also had similar patterns of expression, with $P_{hSurv}$ promoter activity prevailing over the activities of $P_{hTERT}$ and its derivatives, and further potentiation observed for $P_{hSurvD}$ and $P_{mSurv}$ (Group 2 in Fig. 2). The rest of the cell lines assayed (MelCher, MelKor and T47D) showed distinct and individual patterns of promoter variant expression. Clustering of cell lines on the basis of activities of $P_{hTERT}$, $P_{hSurv}$, $P_{mSurv}$ promoters and their variants may reflect common transcription factor repertoires for clustered cell lines and different repertoires between different clusters.

*Comparison of modified and wild type promoter efficiencies.* The data obtained demonstrate that modification of the $P_{hTERT}$ promoter with the synthetic TATA-box or with the minimal CMV promoter improves its performance in tumor cells. The CMV-modified promoter was more active in 9 out of 10 cell lines, while the TATA-modified promoter was more active in 3 out of 10 cell lines; in 1 cell line, the TATA-modified promoter showed slightly lower activity. Importantly, the CMV-modified promoter was more potent compared to the TATA-modified promoter in 7 out of 10 cell lines. Therefore, it is possible to conclude that modification with the minimal CMV promoter is generally preferable to promoters modified

with a TATA-box. Modification of $P_{hTERT}$ with the minimal CMV promoter preserves the intrinsic tumor specificity of the *hTERT* promoter (34); therefore, $P_{hTERT-C}$ appears to be more preferable than the other promoters in this series for the goals of transcriptional targeting of tumor cells.

The human $P_{hSurv}$ promoter was also compared with its modified version, $P_{hSurvD}$, and mouse counterpart, $P_{mSurv}$. The $P_{hSurvD}$ promoter had higher activity in 6 out of 10 cell lines. The mouse promoter was generally more active in human tumor cell lines compared to the human promoter (in 9 out of 10 cell lines). Finally, the $P_{mSurv}$ promoter was more active than the $P_{hSurvD}$ promoter in 4 cell lines. In none of the cell lines did the mouse promoter perform worse than human promoter or its modified version. However the specificity of the mouse promoter toward human tumor cells must be investigated further before its practical utilization to drive therapeutic transgene expression.

*Transgene expression levels directed by the studied promoters are not associated with levels of endogenous hTERT and BIRC5 transcripts*. According to the present results, the relative activities of *hTERT* and survivin gene promoters may vary broadly between tumor cell lines. Thus, we investigated whether relative levels of endogenous *hTERT* or *BIRC5* transcripts could be used to predict the relative activities of the corresponding out-of-gene promoters in gene therapeutic constructions. The relative levels of *hTERT* and *BIRC5* transcripts in cells were determined by semi-quantitative RT-PCR (Fig. 3; Table II). Juxtaposition of the relative levels of *BIRC5* and *hTERT* transcript levels (Table II) with the relative activities of $P_{hTERT}$ and $P_{hSurv}$ (Table I) did not reveal a correlation between them ($r^2$=0.0696; least squares method). Similarly, no correlation was observed when the relative activities of the most potent modified *hTERT* promoter and survivin promoter variant were taken into consideration ($r^2$=0.0762; least squares method). Thus, the relative levels of *hTERT* and *BIRC5* transcripts cannot be used to predict better performance of *hTERT* or survivin promoters (or their derivatives and variations). In agreement with our data, no association was observed between endogenous survivin expression and the level of survivin promoter-driven transgene activity in the previous study (20).

## Discussion

The choice of promoter for the expression of therapeutic genes in tumor gene therapy is of critical importance. Specificity of expression in cancer cells determines the degree of safety of the gene therapeutic approach, whereas promoter strength determines the efficiency of gene expression and, consequently, the effectiveness of treatment. Despite the obvious importance of these parameters, there is virtually no comparison of different promoters used for gene-therapeutic purposes. This renders it difficult to select the promoter that would provide the best outcome when utilized for a certain tumor treatment. In the present study, such a comparison was undertaken to assess the two promoters that are known for their activity in a wide variety of tumors. The results obtained provide several important inferences.

The first inference relates to selection of promoter modification to achieve the most pronounced activity. Taking into account that modification of $P_{hTERT}$ with a minimal CMV promoter generally results in the most potent promoter among the studied *hTERT*-based promoters, and that this modification preserves the intrinsic tumor specificity of the *hTERT* promoter (34), $P_{hTERT-C}$ is the promoter of choice within the analyzed *hTERT* promoter series to be used in transcriptional targeting of tumor cells. The modified human $P_{hSurvD}$ and mouse $P_{mSurv}$ survivin gene promoters are more active than the human $P_{hSurv}$ promoter; however, their tumor specificity requires further investigation before practical utilization.

An important conclusion is associated with the observation that the relative levels of *hTERT* and *BIRC5* transcripts cannot be used to predict better performance of *hTERT* or survivin promoters (or their derivatives and variations). It is likely that the promoters used for the ectopic expression of the reporter gene do not contain the distal or proximal regulatory elements used for endogenous regulation of the corresponding gene expression.

Finally, and most importantly, the two promoters have strikingly different cell specificities. In some cells, *hTERT*-based promoters have predominant activity, whereas survivin-based promoters have their own cells of preference. Only 40% of the cells analyzed maintain activity of both of the promoter series. The levels of promoter activity are strongly different among the cell lines, such that $P_{hTERT}$ activity expressed relative to the activity of $P_{hSurv}$ ranged from 0.17 in A431 cells to 8.56 in MelKor cells, giving 50-fold difference between $P_{hTERT}$ or $P_{hSurv}$ activities among the tumor cell lines. In many cases, promoter activities are complementary to one another such that, if one of them fails to support efficient transgene expression in a tumor, the other can be used instead. However, the necessity of selecting the most active promoter between $P_{hTERT}$ and $P_{hSurv}$ complicates the use of the separate promoters and makes highly desirable the creation of a combined promoter composed from elements derived from *hTERT*- and survivin-based promoters so that such a hybrid promoter would be equally active in different cells. Otherwise, owing to the complementary nature of survivin and *hTERT* promoter activities observed in the present study, the simultaneous use of survivin- and *hTERT*-driven therapeutic transgene vectors emerges as a feasible option to ensure efficient transgene expression in a variety of cancer cells.

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