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Invited review

Telomere and telomerase as targets for anti-cancer and regeneration therapies[1]

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Abstract

Telomerase is a ribonucleoprotein that directs the synthesis of telomeric sequence. It is detected in majority of malignant tumors, but not in most normal somatic cells. Because telomerase plays a critical role in cell immortality and tumor formation, it has been one of the targets for anti-cancer and regeneration drug development. In this review, we will discuss therapeutic approaches based mainly on small molecules that have been developed to inhibit telomerase activity, modulate telomerase expression, and telomerase directed gene therapy.

Introduction

Telomeres are specialized protein-DNA structures at the end of eukaryotic chromosomes. They protect chromosomes from end-to-end fusion and nuclease degradation. In humans, telomeres consists of approximately 4–14 kb of TTAGGG duplex repeats and 150–200 bases of single-stranded DNA overhang running 5' to 3' toward the end of chromosome[1]. Because of the end replication problem[2,3], telomeres in human cells erode by approximately 100 bp with each cell division[4]. Telomerase is the key enzyme for the stabilization of telomere by adding TTAGGG repeats to telomere ends[5]. It is a ribonucleoprotein that utilizes its RNA component as the template to synthesis telomere repeats. In humans, telomerase activity is not detected in most somatic cells[6,7]. Progressive telomere shortening after each cell division leads to cellular senescence after 60–80 population doublings[4]. Escaping senescence leads to further shortening of telomeres and can eventually cause cells to enter crisis and cell death[8]. Cells at this stage appear aneuploidy because telomere loss induces chromosome instability through the breakage/fusion/bridge cycles[9]. Few cells stabilize telomere length through activating telomerase activity[10]. Indeed, telomerase activity is detected in approximately 90% to 95% of human immortal cell lines and up to 85% of cancers[6,7]. In the remaining 5% to 10% immortal cells, telomerase-independent telomere maintenance mechanism, also known as the alternative lengthening of telomeres (ALT), were used to maintain telomere length[11]. These cells could then divide with great capacity.

Because telomerase is an essential component for the proliferation of cancer cells, targeting telomere and telomerase has drawn the interests of scientists in anti-cancer and regeneration drug development. In the present study, we discuss various approaches used including the small molecules that have been developed using telomere and telomerase as targets for chemotherapeutic developments.

Inhibition of telomerase activity

Although tumors are caused by mutations that activate oncogenes and repress tumor suppressor genes, they still need to overcome telomere-dependent senescence for their indefinite divisions. Because telomerase activity is crucial for supporting indefinite proliferation of most tumor cells, selective inhibition of telomerase activity only limits the proliferation capacity of tumor cells[12]. In principle, a telomerase-specific inhibitor is expected to affect telomere maintenance. It has been proposed that a telomerase inhibitor can be utilized as complementary therapy in cancer chemotherapeutics[12,13]. However, more and more studies indicate that telomerase also plays an important role in the telomere-cap-

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ping function[14]. Inhibition of telomerase can affect the survival of cancer cells. Indeed, several published reports have found that the inhibition of telomerase can cause apoptosis without long-term treatment of the cells[15–17]. Thus, it is expected that the inhibition of telomerase activity in cells produces different effects depending on the means of inhibition. Because telomerase activities are also presented in human germline cells, stem cells, peripheral blood mononuclear cells, and normal fibroblasts[6,18,19], it is likely that telomerase inhibition also affects these cells. The potential risks of telomerase inhibition have to be carefully evaluated in these cells.

Targeting telomerase Telomerase is a unique reverse transcriptase consisting of two major components, the RNA template (*hTR*) and the catalytic subunit (hTERT). Both components have been used as targets for telomerase inhibition. The first successful case was reported in 1995 where antisense RNA against the first 185 nucleotides of the *hTR* molecule was introduced into HeLa cells and caused progressive telomere shortening and eventually cell crisis[20]. Similarly, short peptide nucleic acid (PNA) or 2'-*O*-methyl-RNA (2'-*O*-meRNA) oligomers with enhanced binding properties to *hTR* efficiently inhibit telomerase activity, and lead to progressive telomere shortening in immortal breast epithelial cells[21]. Synthetic oligonucleotides applying 2–5A (5'-phosphorylated 2'-5'-linked oligoadenylate)-linked antisense approaches were used to degrade *hTR* and caused apoptosis in several cancer models including glioma[15]. Utilizing ribozymes to cleave *hTR* has also been reported in several published studies[13,22]. These small catalytically-active RNA molecules cleave their RNA substrate in a sequence-dependent manner. Interestingly, the inhibition of telomerase activity using hammerhead ribozyme against *hTR* appears to sensitize the breast epithelial cells to topoisomerase inhibitors[13]. Another approach is to target the catalytic subunit of telomerase hTERT. A dominant negative mutant of hTERT was identified that caused complete inhibition of telomerase activity, telomere shortening, and increased cell apoptosis when introduced into cancer cells[16,17]. This dominant negative-hTERT also reduced tumorigenicity in nude mice[16].

Even though antisense, ribozyme, and dominant negative approaches showed promising results in inhibiting telomerase activity, these approaches were less applicable because the techniques for effective and convenient delivery of RNA or proteins were not available for clinical settings. Thus, small molecule compounds that inhibit telomerase activity appear to be more suited. There were several types of compounds identified, including nucleotide analogs that inhibit the catalytic activity of the enzyme and non-nucleotide analogs that have inhibitory effects less characterized. As these molecules have been extensively reviewed recently[23,24], they will not be discussed further here. However, it is worthy of noting that compound BIBR1532 was shown to inhibit telomerase non-competitively[25] and cause telomere shortening and senescence in cancer cells[26]. Moreover, cancer cells pretreated with BIBR1532 showed a reduced tumorigenic potential in the mouse xenograft model[26]. With the IC_{50} value at nanomolar concentration, BIBR1532 and several other compounds should have the potential for further developments[26–28].

Targeting telomeres In humans, the G-rich telomeric DNA tails are capable of forming a planar structure, termed G-quadruplexes, through non-Watson-Crick Hoogsteen hydrogen bonding *in vitro*[29]. A recent study has detected the G-quadruplex-induced fluorescence in telomeres of metaphase chromosomes using a G-quadruplex-selective fluorescent compound BMVC [3,6-*bis*(1-methyl-4-vinylpyridinium) carbazole diiodide], providing the first evidence for the presence of G-quadruplexes inside human cells[30]. In addition to telomeres, the G-quadruplex structure was also reported in the transcriptional regulatory region of several important oncogenes that their expression could be regulated by them[31]. Thus, even though telomeres in human cells were shown to form T-looped structures[32], it appears that human telomeres could also form G-quadruplex structures inside cells. The function of G-quadruplex structure in telomeres is not clear. However, because G-quadruplexes formed by telomeric DNA sequences were not the substrates for telomerase[33], the G-quadruplex structure might have a role in telomere maintenance and transcriptional regulation of oncogene expression. Agents that stimulate the formation or stabilize G-quadruplexes become important targets for drug design.

Several researchers have adopted a structure-based design and synthesis approach to identify lead compounds that interact with G-quadruplexes[24]. Successful lead compounds that were identified include derivatives of proflavins[28], porphyrins[34], acridines[35], anthraquinones[36], triazines[37], and carbazoles[27]. All of these identified compounds have planar aromatic rings. Molecular modeling studies have indicated that these planar structures bind to the G-quadruplexes through a series of interactions to the planar and loop structures of G-quadruplexes[28,38,39]. These interactions stabilize the structure of G-quadruplexes and increase the melting temperature upon binding. For example, a carbazole derivative BMVC increases the melting temperature (Tm) of G-quadruplexes formed by human telomeric DNA by as much as 13 ºC[40]. It is interesting to note that these planar compounds appear to be very effective in affecting telomerase

as several of these compounds have the IC_{50} at the range of nanomolar concentration[27,28,37].

Modulation of telomerase expression

Direct inhibition of telomerase at the activity level provides a simpler mean to target telomerase for cancer therapy. In recent years, several reports have also attempted to target the expression of telomerase at the gene level. Repression of telomerase expression in cancer cells would have applications in anti-cancer chemotherapies whereas activation of telomerase expression in normal cells would have applications in regeneration therapies.

Repressing hTERT expression in cancer cells In human cells, the expressing of telomerase catalytic enzyme hTERT correlates well with the telomerase activity[41]. It also appears that regulation at the transcriptional level was the most important step for hTERT expression[42] even though regulation at the splicing[43,44], post-translational modification[45–48], or subcellular localization[49] were also reported. Thus, targeting hTERT transcription is the focus for developing agents that repress hTERT expression. However, there were only a limited number of reports targeting hTERT expression. Retinoic acid represents the most characterized small molecule in this category. Retinoic acid at the micromolar concentration down-regulates telomerase activity in human leukemia cells[50,51]. Long-term treatment of leukemia cells with retinoic acid leads to telomere shortening and eventually cell death[50]. The specificity of retinoic acid is a concern in future drug development. An ideal compound should only affect the expression of the hTERT gene. Nevertheless, repressing hTERT expression is an effective way to limit the proliferation capacity of cancer cells. Recently, a cell-based system was developed that enables the screening of small molecule compounds for repressing hTERT expression[52]. The hTERT promoter was ligated downstream to a reporter gene, GFP or SEAP (secreted alkaline phosphatase), and introduced into human cancer cells. The expression of hTERT could then be monitored by the reporter genes. A series of anthraquinone derivatives were tested for their ability to repress hTERT expression using this cell-based system[52]. Even though anthraquinone derivatives did not repress hTERT expression, it is anticipated that small molecule compounds that repress hTERT expression could be screened and identified using this approach.

Activating hTERT expression in normal cells While telomerase inhibition or hTERT repression could have applications in anticancer therapeutics, telomerase activation could serve as a mean to extend the lifespan of normal cells and to treat degenerative diseases. For example, liver cirrhotic pathology is caused by continual hepatocyte destruction over many years. The end-stage of cirrhosis is characterized by extensive fibrotic replacement and cessation of hepatocyte proliferation. In a mouse experimental liver cirrhosis model, the pathology is alleviated through activation of telomerase activity[53]. Also in Werner syndrome cells, the accelerated aging phenotype is reversed by telomerase activation[54]. Moreover, the life span of human bone marrow stromal stem cells are achieved by telomerase expression[55,56]. These *ex vivo* expanded stem cells could have profound applications in, for instance, tissue engineering.

Ectopically expressed hTERT by introducing a virus promoter-driven hTERT gene into normal human cells has been found to be very effective in increasing hTERT mRNA expression and telomerase activity, and extending the life span of normal cells[10,57]. For example, the replication capacity of skin fibroblasts[10] and adult mesenchymal stem cells[58] could be expanded with the introduction of a viral promoter driven hTERT gene. However, given the role of telomerase in cancer and the introduced DNA which integrates randomly into chromosomes, this type of approach might cause some risks. Although some reports indicated that ectopic expression of telomerase did not cause transformation phenotypes or cancer-associated changes[59,60], more and more studies raise concerns about the future application of cells immortalized by ectopic hTERT expression in normal cells. Telomerase overexpression in mice has been found to increase epidermal tumors and promote mammary carcinomas[58,61,62] and that expressing hTERT in human mesenchymal stem cells renders these cells tumorigenic[58]. Thus, the ideal situation would be controlled expression of hTERT in target cells to avoid unnecessary side-effects. One approach is using small molecules that activate telomerase upon addition and return to the repressed state upon removal which might provide the solution for controlled expression of hTERT. This approach also avoids the uncertainty concerning the integration of DNA into chromosomes.

There are several molecules which have been identified that affect hTERT expression through inhibiting DNA methylation or histone deacetylation. Normal human fibroblast treated with 5-azacytidine (5-AZC) can cause demethylation of the CpG islands within hTERT promoter. It then turns on hTERT mRNA expression and activates telomerase activity[63,64]. Similarly, histone deacetylase inhibitor tricostain A (TSA) causes activation of hTERT mRNA expression and telomerase activity in human normal cells[65,66]. However, because both DNA methylation and histone deacetylation are general mechanisms in controlling gene expression in human cells, the treatment of 5-AZC or TSA would have

broad effects on cellular gene expressions. To achieve selective activation of hTERT expression, a more specific promoter-targeted agent is desired. Sequence analysis of hTERT promoter reveals an estrogen response element (ERE) located upstream to the transcription start site. The function of ERE is established in normal human ovary epithelium cells where the addition of 17â-estradiol activates the expression of hTERT and telomerase activity[67]. Thus, telomerase activity could be activated by estrogen in cells with estrogen receptors. However, since estrogens also activate the telomerase activities in cancer cells with estrogen receptor[67–69], the applicability of estrogen or its analogs in clinics remains to be evaluated. Similar to the approach used in identifying small molecule compounds, a cell-based reporter system was developed in normal human cells that enabled the identification of several *bis*-substituted derivatives of anthraquinones that activate hTERT[52]. Unlike inhibitors of DNA methylation or histone deacetylation, the repression of hTERT expression by these anthraquinones appears to be specific as they do not activate the expression of reporter gene driven by a virus promoter. Using a similar cell-based chemical screening strategy, compound CGK1026 was also identified to activate hTERT expression in normal human fibroblasts[70]. CGK1026 activates hTERT expression by affecting the interaction between E2F-pocket protein and histone deacetylase. The effect of CGK1026 on other promoters is still unclear.

Telomerase-directed tumor gene therapy

One of the major goals in anticancer therapies is to target toxic agents to tumor cells specifically to minimize the effects toward normal cells. The specific expression of telomerase hTERT in most types of tumors provides a good discrimination between cancer and normal cells[6,7]. Deletion analysis of the hTERT promoter reveals a core promoter region located approximately 200 bp upstream of the transcription start site, and is sufficient to confer its specific expression in cancer cells[71]. The property of hTERT promoter has been applied to restrict the expression of therapeutic genes in tumors. The therapeutic genes utilized include apoptosis-inducing[72–74], toxin-encoding[75], chemotherapeutic sensitizer[71], xenoantigen[76] genes, or genes used in gene-directed enzyme prodrug therapy (GDEPT)[77,78]. These hTERT promoter-driven therapeutic genes were introduced into tumor cells through liposome- or virus-mediated pathways. As expected from the expression pattern of telomerase, this type of approach selectively kills virtually all types of telomerase-positive cancer cells without affecting the viability of telomerase-negative cells in both cellular and animal xenograft models. Thus, hTERT directed tumor gene therapy appears to be a promising approach in treating telomerase-positive tumors. Because hTERT expression varies in different telomerase-positive cells[72–74], it is anticipated that telomerase-directed tumor gene therapy would work better in cancer cells with high levels of hTERT expression.

Summary

Because of the unique property of telomere and telomerase in cancer and the aging process, they have been the targets for new drug developments toward anticancer or regenerative disease therapeutics. In addition to various approaches that have been described here, other approaches based on the property of telomerase have also been reported. For example, hTERT has been reported as a tumor-associated antigen in a wide range of tumors. These hTERT-derived tumor antigens could be recognized by cytotoxic T lymphocytes that could then be applied in designing anticancer immunotherapeutic strategies[79,80]. It is anticipated that other novel approaches based on telomere functions will be developed.

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References

1 Zakian VA. Telomeres: beginning to understand the end. Science 1995; 270: 1601–7.
2 Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymatic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol 1973; 41: 181–90.
3 Watson JD. Origin of concatemeric DNA. Nature (New Biol) 1972; 239: 197–201.
4 Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990; 345: 458–60.
5 Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 1989; 59: 521–9.
6 Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, *et al*. Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266: 2011–5.
7 Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997; 33: 787–91.
8 Shay JW, Roninson IB. Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene 2004; 23: 2919–33.
9 Gisselsson D, Jonson T, Petersen A, Strombeck B, Dal Cin P, Hoglund M, *et al*. Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors. Proc Natl Acad Sci USA 2001; 98: 12683–8.
10 Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, *et al*. Extension of life-span by introduction of telomerase into normal human cells. Science 1998; 279: 349–52.
11 Henson JD, Neumann AA, Yeager TR, Reddel RR. Alternative

lengthening of telomeres in mammalian cells. Oncogene 2002; 21: 598–610.
12 Shay JW, Wright WE. Telomerase: a target for cancer therapeutics. Cancer Cell 2002; 2: 257–65.
13 Ludwig A, Saretzki G, Holm PS, Tiemann F, Lorenz M, Emrich T, *et al*. Ribozyme cleavage of telomerase mRNA sensitizes breast epithelial cells to inhibitors of topoisomerase. Cancer Res 2001; 61: 3053–61.
14 Blackburn EH. Switching and signaling at the telomere. Cell 2001; 106: 661–73.
15 Kondo S, Kondo Y, Li G, Silverman RH, Cowell JK. Targeted therapy of human malignant glioma in a mouse model by 2-5A antisense directed against telomerase RNA. Oncogene 1998; 16: 3323–30.
16 Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, *et al*. Inhibition of telomerase limits the growth of human cancer cells. Nature Med 1999; 5: 1164–70.
17 Zhang X, Mar V, Zhou W, Harrington L, Robinson MO. Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes Dev 1999; 13: 2388–99.
18 Bodnar AG, Kim NW, Effros RB, Chiu CP. Mechanism of telomerase induction during T cell activation. Exp Cell Res 1996; 228: 58–64.
19 Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, *et al*. Telomerase maintains telomere structure in normal human cells. Cell 2003; 114: 241–53.
20 Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, *et al*. The RNA component of human telomerase. Science 1995; 269: 1236–41.
21 Herbert BS, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW, *et al*. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. Proc Natl Acad Sci USA 1999; 96: 14276–81.
22 Yokoyama Y, Takahashi Y, Shinohara A, Lian Z, Wan X, Niwa K, *et al*. Attenuation of telomerase activity by a hammerhead ribozyme targeting the template region of telomerase RNA in endometrial carcinoma cells. Cancer Res 1998; 58: 5406–10.
23 Rezler EM, Bearss DJ, Hurley LH. Telomeres and telomerases as drug targets. Curr Opin Pharmacol 2002; 2: 415–23.
24 Neidle S, Parkinson G. Telomere maintenance as a target for anticancer drug discovery. Nat Rev Drug Discov 2002; 1: 383–93.
25 Pascole E, Wenz C, Lingner J, Hauel N, Priepke H, Kauffmann I, *et al*. Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. J Biol Chem 2002; 277: 15566–72.
26 Damm K, Hemmann U, Garin-Chesa P, Hauel N, Kauffmann I, Priepke H, *et al*. A highly selective telomerase inhibitor limiting human cancer cell proliferation. EMBO J 2001; 20: 6958–68.
27 Chang CC, Kuo IC, Lin JJ. A novel carbazole derivative, BMVC: a potential antitumor agent and fluorescence marker of cancer cells. Chem Biodivers 2004; 1: 1377–84.
28 Read M, Harrison RJ, Romagnoli B, Tanious FA, Gowan SH, Reszka AP. Structure-based design of selective and potent G-quadruplex-mediated telomerase inhibitors. Proc Natl Acad Sci USA 2001; 98: 4844–9.
29 Sen D, Gilbert W. The structure of telomeric DNA: DNA quardruplex formation. Curr Opin Struct Biol 1991; 1: 435–8.
30 Chang CC, Kuo IC, Ling IF, Chen CT, Chen HC, Lou PJ. Detection of quadruplex DNA structures in human telomeres by a fluorescent carbazole derivative. Anal Chem 2004; 76: 4490–4.
31 Siddiqui-Jain A, Grand CL, Bearss DJ, Hurley LH. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc Natl Acad Sci USA 2004; 99: 11593–8.
32 Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, *et al*. Mammalian telomeres end in large duplex loop. Cell 1999; 97: 503–14.
33 Zahler AM, Williamson JR, Cech TR, Prescott DM. Inhibition of telomerase by G-quartet DNA structure. Nature 1991; 350: 718–20.
34 Wheelhouse RT, Sun D, Han H, Han FX, Hurley LH. Cationic porphyrins as telomerase inhibitors: the interaction of tetra-(*N*-methyl-4-pyridyl)porphine with quadruplex DNA. J Am Chem Soc 1998; 120: 3261–2.
35 Heald RA, Modi C, Cookson JC, Hutchinson I, Laughton CA, Gowan SM, *et al*. Antitumor polycyclic acridines. 8. Synthesis and telomerase-inhibitory activity of methylated pentacyclic acridinium salts. J Med Chem 2002; 45: 590–7.
36 Sun D, Thompson B, Cathers BE, Salazar M, Kerwin SM, Trent JO, *et al*. Inhibition of human telomerase by a G-quadruple-interactive compound. J Med Chem 1997; 40: 2113–28.
37 Riou JF, Guittat L, Mailliet P, Laoui A, Renou E, Petitgenet O, *et al*. Cell senesscence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. Proc Natl Acad Sci USA 2002; 99: 2672–7.
38 Perry PJ, Read MA, Davies RT, Gowan SM, Reszka AP, Wood AA, *et al*. 2,7-Disubstituted amidofluorenone derivatives as inhibitors of human telomerase. J Med Chem 1999; 42: 2679–84.
39 Cairns D, Michalitsi E, Jenkins TC, Mackay SP. Molecular modeling and cytotoxicity of substituted anthraquinones as inhibitors of human telomerase. Bioorgan Med Chem 2002; 10: 803–7.
40 Chang CC, Wu JY, Chang TC. A carbazole derivative synthesis for stabilizing the quadruplex structure. J Chin Chem Soc 2003; 50: 185–8.
41 Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, *et al*. Telomerase catalytic subunit homologs from fission yeast and human. Science 1997; 277: 955–9.
42 Ducrest AL, Szutorisz H, Lingner J, Nabholz M. Regulation of the human telomerase reverse transcriptase gene. Oncogene 2002; 21: 541–52.
43 Ulaner GA, Hu JF, Vu TH, Oruganti H, Giudice LC, Hoffman AR. Regulation of telomerase by alternate splicing of human telomerase reverse transcriptase (hTERT) in normal and neoplastic ovary, endometrium and myometrium. Int J Cancer 2000; 85: 330–5.
44 Yi X, White DM, Aisner DL, Baur JA, Wright WE, Shay JW. An alternate splicing variant of the human telomerase catalytic subunit inhibits telomerase activity. Neoplasia 2000; 2: 433–40.
45 Ku WC, Cheng AJ, Wang TCV. Inhibition of telomerase activity by PKC inhibitors on human nasopharyngeal cancer cells in culture. Biochem Biophys Res Commun 1997; 241: 730–6.
46 Li H, Zhao LL, Funder JW, Liu JP. Protein phosphatase 2A inhibits nuclear telomerase activity in human breast cancer cells. J Biol Chem 1997; 272: 16729–32.
47 Li H, Zhao L, Yang Z, Funder JW, Liu JP. Telomerase is controlled by protein kinase Ca in human breast cancer cells. J Biol Chem 1998; 273: 33436–42.
48 Kang SS, Kwon T, Kwon DY, Do SI. Akt protein kinase enhances human telomerase activity through phosphorylation of telo-merase reverse transcriptase subunit. J Biol Chem 1999; 274: 13085–90.
49 Wong JMY, Kusdra L, Collins K. Subnuclear shuttling of human telomerase induced by transformation and DNA damage. Nature

Cell Biol 2002; 4: 731–6.
50 Pendino F, Flexor M, Delhommeau F, Buet D, Lanotte M, Segal-Bendirdjian E. Retinoids down-regulate telomerase and telomere length in a pathway distinct from leukemia cell differentiation. Proc Natl Acad Sci USA 2001; 98: 6662–7.
51 Mano Y, Shimizu T, Tanuma S, Takeda K. Synergistic down-regulation of telomerase activity and hTERT mRNA expression by combination of retinoic acid and GM-CSF in human myeloblastic leukemia ML-1 cells. Anticancer Res 2000; 20: 1649–52.
52 Huang HS, Chiou JF, Fong Y, Hou CC, Lu YC, Wang JY, *et al*. Activation of human telomerase reverse transcriptase expression by some new symmetrical *bis*-substituted derivatives of the anthraquinone. J Med Chem 2003; 46: 3300–7.
53 Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. Science 2000; 287: 1253–8.
54 Wyllie FS, Jones CJ, Skinner JW, Haughton MF, Wallis C, Wynford-Thomas D, *et al*. Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts. Nat Genet 2000; 24: 16–7.
55 Shi S, Gronthos S, Chen S, Reddi A, Counter CM, Robey PG, *et al*. Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. Nat Biotechnol 2002; 20: 587–91.
56 Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenderup K, Rattan SI, *et al*. Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol 2002; 20: 592–6.
57 Counter CM, Meyerson M, Eaton EN, Ellisen ZW, Caddle SD, Haber DA, *et al*. Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase. Oncogene 1998; 16: 1217–22.
58 Serakinci N, Guldberg P, Burns JS, Abdallah B, Schrodder H, Jensen T, *et al*. Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 2004; 23: 5095–8.
59 Jiang XR, Jimenez G, Chang E, Kaur KJ, Yan Y, Wilson KS, *et al*. Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nat Genet 1999; 21: 111–4.
60 Morales CP, Holt SE, Ouellette M, Kaur KJ, Yan Y, Wilson KS, *et al*. Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 1999; 21: 115–8.
61 Gonzalez-Suarez E, Samper E, Ramirez A, Flores JM, Martin-Caballero J, Jorcano JL, *et al*. Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. EMBO J 2001; 20: 2619–30.
62 Artandi SE, Alson S, Tietze MK, Sharpless NE, Yes, Greenberg RA, *et al*. Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci USA 2002; 99: 8191–6.
63 Devereux TR, Horikawa I, Anna CH, Annab LA, Afshari CA, Barrett JC, *et al*. DNA methylation analysis of the promoter region of the human telomerase reverse transcriptase (hTERT) gene. Cancer Res 1999; 59: 6087–90.
64 Dessain SK, Yu H, Reddel RR, Beijersbergen RL, Weinberg RA. Methylation of the human telomerase gene CpG island. Cancer Res 2000; 60: 537–41.
65 Cong YS, Bacchetti S. Histone deacetylation is involved in the transcriptional repression of hTERT in normal human cells. J Biol Chem 2000; 275: 35665–8.
66 Takakura M, Kyo S, Sowa Y, Wang Z, Yatabe N, Maida Y, *et al*. Telomerase activation by histone deacetylase inhibitor in normal cells. Nucleic Acids Res 2001; 29: 3006–11.
67 Misiti S, Nanni S, Fontemaggi G, Cong YS, Wen J, Hirte HW, *et al*. Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells. Mol Cell Biol 2000; 20: 3764–71.
68 Kyo S, Takakura M, Kanaya T, Cong YS, Wen J, Hirte HW, *et al*. Estrogen activates telomerase. Cancer Res 1999; 59: 5917–21.
69 Wang Z, Kyo S, Takakura M, Tanaka M, Yatabe N, Maida Y, *et al*. Progesterone regulates human telomerase reverse transcriptase gene expression via activation of mitogen-activated protein kinase signaling pathway. Cancer Res 2000; 60: 5376–81.
70 Won J, Chang S, Oh S, Kim TK. Small-molecule-based identification of dynamic assembly of E2F-pocket protein-histone deacetylase complex for telomerase regulation in human cells. Proc Natl Acad Sci USA 2004; 101: 11328–33.
71 Nakamura M, Kyo S, Kanaya T, Yatabe N, Maida Y, Tanaka M, *et al*. hTERT-promoter-based tumor-specific expression of MCP-1 effectively sensitizes cervical cancer cells to a low dose of cisplatin. Cancer Gene Ther 2004; 11: 1–7.
72 Gu J, Kagawa S, Takakura M, Kyo S, Inoue M, Roth JA, *et al*. Tumor-specific transgene expression from the human telomerase reverse transcriptase promoter enables targeting of the therapeutic effects of the Bax gene to cancers. Cancer Res 2000; 60: 5359–64.
73 Komata T, Kondo Y, Kanzawa T, Hirohata S, Koga S, Sumiyoshi H, *et al*. Treatment of malignant glioma cells with the transfer of constitutively active caspase-6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. Cancer Res 2001; 61: 5796–802.
74 Koga S, Hirohata S, Kondo Y, Komata T, Takakura M, Inoue M, *et al*. A novel telomerase-specific gene therapy: gene transfer of caspase-8 utilizing the human telomerase catalytic subunit gene promoter. Hum Gene Ther 2000;11: 1397–406.
75 Abdul-Ghani R, Ohana P, Matouk I, Ayesh S, Ayesh B, Laster M, *et al*. Use of a transcriptional regulatory sequences of telomerase (hTER and hTERT) for selective killing of cancer cells. Mol Ther 2000; 2: 539–44.
76 Sawada T, Yamada O, Yoshimura N, Hatori K, Fuchinoue S, Teraoka S, *et al*. Xenoantigen, an aGal epitope-expression construct driven by the hTERT-promoter, specifically kills human pancreatic cancer cell line. Cancer Cell Int 2002; 2: 14–20.
77 Majumdar AS, Hughes DE, Lichtsteiner SP, Wang Z, Lebkowski JS, Vasserot AP, *et al*. The telomerase reverse transcriptase promoter drives efficacious tumor suicide gene therapy while preventing hepatotoxicity encountered with constitutive promoters. Gene Ther 2001; 8: 568–78.
78 Song JS, Kim HP, Yoon WS, Lee KW, Kim MH, Kim KT, *et al*. Adenovirus-mediated suicide gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter induced apoptosis of ovarian cancer cell line. Biosci Biotechnol Biochem 2003; 67: 2344–50.
79 Vonderheide RH, Hahn WC, Schultze JL, Nadler LM. The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity 1999; 10: 673–9.
80 Vonderheide RH. Telomerase as a universal tumor-associated antigen for cancer immunotherapy. Oncogene 2002; 21: 674–9.