Commentary

Complex Regulation of Telomerase Activity

Implications for Cancer Therapy

Kojo S. J. Elenitoba-Johnson

From the Department of Pathology, University of Utah School of Medicine, Salt Lake City; and the Associated and Regional University Pathologists Institute for Clinical and Experimental Pathology, Salt Lake City, Utah

Telomerase activity is a marker of malignancy. The observation that its activity is present in 85 to 90% of all human tumors but not in normal nonneoplastic cells¹ has made telomerase a superb target not only for the diagnosis of malignancy, but also for the development of novel therapeutic agents. For neoplastic cells to maintain sustained proliferation beyond the cellular senescent phase, cells must either reactivate telomerase or inhibit repressors of telomerase activity. Thus, the mechanisms regulating telomerase activity are of great interest and are the focus of the work by Lin and colleagues,² in this issue of *The American Journal of Pathology*.

Telomeres are repetitive short DNA sequences located at the ends of linear chromosomes.³ The telomeres contain 5- to 15-kb pairs of repeating TTAGGG sequences that shorten by 50 to 200 bp with each cell division. They protect the chromosomes from DNA degradation, end-to-end fusions, rearrangements, and chromosome loss, and maintain nuclear structure.^{4,5} Without mechanisms for maintenance of telomere length, the chromosomal ends would be recognized and repaired with subsequent fusion and chromosomal instability.⁶ Normal cultured human cells have a finite replication potential reaching a discrete point at which cell growth ceases. This stage is also characterized by the shortening of a few telomeres to a size that leads to growth arrest or cellular senescence. The activation of the enzyme telomerase can induce an immortal state by stabilizing the length of its telomeres.

The major components of the telomerase ribonucleoprotein holoenzyme are the human telomerase RNA (hTR),⁷ telomerase-associated protein 1 (hTEP1),⁸ and the human telomerase reverse transcriptase (hTERT) catalytic subunit.⁹ The hTR RNA is 445 nucleotides long and contains an 11-bp sequence (5'-CUAACCCUAAC-3') coding for the telomere repeats of (TTAGGG)_n¹⁰ that

serves as the template on which telomeric repeats are added to the chromosome. The cellular activity of telomerase is determined by the presence or absence of hTERT, whereas all human somatic cells constitutively express hTR. TEP1 is a protein that binds to both hTR and hTERT and maintains the tertiary and/or quaternary structure of the telomerase holoenzyme.⁸

Both *in vitro* and *in vivo* studies have shown that hTERT is the major determinant of telomerase activity.¹¹ The notion that dysregulation of hTERT gene expression is a key factor in carcinogenesis is supported by the observation that hTERT transcripts are low or undetectable in most human somatic cells but readily detectable in transformed counterparts.¹² Thus, were it not for activation of telomerase, tumor cells would either undergo terminal growth arrest, or die. According to proponents of the tumor suppressor function of telomere shortening, inhibition of this inappropriately expressed enzyme would render the cells unable to maintain their telomeric DNA, resulting in tumor stasis and/or regression. This has fueled the recent enthusiasm for development of a class of drugs that would target telomerase in tumor cells.

Recent developments in this field have complicated the previously held views. Initial studies had demonstrated telomerase activity as a marker of germ cells or malignant cells. The study of hTERT expression in lymphoid cells during development, differentiation, and activation supported the notion that telomerase activity can be highly regulated. Activation of peripheral blood B¹³ and T lymphocytes increased both the levels of hTERT transcripts and telomerase activity.¹⁴⁻¹⁷ Moreover, different lymphoid lineages may have different rates of telomere shortening with CD4⁺ T cells having the longest average telomeres followed by B cells with CD8⁺ T cells having the shortest telomeres suggesting that these lym-

Supported by a grant from the National Institutes of Health (no. CA 83984).

Accepted for publication June 4, 2001.

Address reprint requests to Kojo S. J. Elenitoba-Johnson, M.D., Division of Anatomic Pathology, University of Utah School of Medicine, 50 North Medical Dr., Salt Lake City, Utah 84132. E-mail: kojo.elenitobaj@path.utah.edu.

phocyte populations have different replicative histories *in vivo*.¹⁸

Study of the promoter region of hTERT demonstrated that it is inactive in normal human somatic cells but becomes activated during cell immortalization.¹⁹ Sequence analysis of the hTERT promoter²⁰ also revealed binding sites for several transcription factors suggesting that hTERT expression is subject to multiple levels of control and is regulated by different factors in different cellular contexts. Specifically, a 59-bp region required for maximal promoter activity contained a potential MYC oncoprotein binding-site (E-box), and co-transfection of a *c-myc* expression plasmid markedly enhanced its promoter activity, whereas anti-sense *c-myc* oligonucleotides resulted in down-regulation of telomerase activity in human leukemic cell lines.²¹ These findings suggest that activation of oncogenes or loss of tumor suppressor gene function may serve to override the strict expression of hTERT in nonneoplastic cells. Indeed, studies of the regulation of hTERT have shown that a variety of oncogenes positively regulate telomerase activity whereas tumor suppressor genes accomplish the converse. Oncogenic variants of HPV E6 protein induced strong telomerase activity in mammary epithelial cells whereas weakly oncogenic variants induced significantly less telomerase activity.²² The C-MYC oncoprotein mediated the increase in telomerase activity in human fibroblasts by directly activating the transcription of hTERT gene.^{23,24} Other oncogenes such as *SV40*, *K-ras*,^{24,25} protein kinase C,²⁵ *bcl-2*,²⁶ *c-Abl*,²⁷ and *Akt*²⁸ also induced hTERT expression.

Conversely, introduction of tumor suppressor genes such as pRB,²⁹ autocrine transforming growth factor- β ,³⁰ and p21 Waf-1³¹ into tumor cell lines resulted in a repression of telomerase activity. In addition, elements in chromosome 3 seem to be important in telomerase suppression. Introduction of chromosome 3 into telomerase-positive renal carcinoma cells³² or breast carcinoma cells³³ resulted in the repression of hTERT expression, down-regulation of telomerase activity, up-regulation of telomerase shortening, and cessation of cell growth.

Recent studies have suggested that telomerase activity can also be regulated by alternative splicing of hTERT transcripts, with resulting loss of enzymatic activity in fetal organ development³⁴ and in some tumor cells.³⁵ In addition, the amplification of the hTERT gene has been observed in many primary human tumors and cell lines.³⁶

Increasing the complexity of the story are the data demonstrating the existence of posttranscriptional modes of telomerase regulation. A highly GC-rich content of the 5'-end of the hTERT cDNA spanning to the 5'-flanking region and intron 1, characteristic of a CpG island has been identified.²⁰ The functional significance of the 72 CpG sites within the promoter has been confirmed by the observation that the expression of hTERT can be modulated at the epigenetic level by promoter methylation,³⁷ although it is not responsible for repressing hTERT expression in most telomerase-negative cells. Treatment of cells with the demethylating agent 5-aza-2'-deoxycytidine induced the expression of hTERT,³⁸ suggesting a potential role for DNA methylation in the negative regulation of hTERT. In addition, the histone deacetylase

inhibitor Trichostatin A, an agent associated with cellular differentiation reduced the levels of telomerase activity in human liver cancer cells without affecting transcription levels of the hTERT.³⁹ This observation lends support for the role of telomerase in maintaining nucleoprotein stability. It also suggests that telomerase may have a role in histone deacetylase inhibitor-based treatments for acute myeloid⁴⁰ and lymphoid⁴¹ leukemias.

Posttranslational modifications and protein-protein interactions also seem to modulate telomerase activity. Telomerase is slowly metabolized with a half-life of more than 24 hours,⁴² because of posttranslational assembly, maintenance, and conformational changes required by the components of the holoenzyme. Identification of a large number of WD-40 repeats at the carboxy-terminal region of hTERT protein putatively implicated in mediating protein-protein interactions, suggests that it may interact with molecules such as transcription factors, proteasomal proteins that regulate telomerase function.⁹ Identification of telomerase interactive proteins by affinity chromatography with synthetic peptides derived from the sequences of hTERT has yielded interesting results. Screening of nuclear proteins from human breast cancer cells identified p53 tumor suppressor protein that co-immunoprecipitated with hTERT and also inhibited telomerase activity *in vitro*.⁴³ The inhibitory effect of p53 on telomerase was abrogated by TEIPP1, a peptide capable of inhibiting telomerase activity suggesting a direct interaction between p53 and hTERT. These data suggest that telomerase may be a downstream target of p53 with its activity being modulated by the tumor suppressor gene. This is consistent with the report that p53-specific mutations are involved in telomerase activation secondary to sun exposure⁴⁴ and that expression of a p53 mutant may modulate telomerase activation in certain genetic contexts.⁴⁵ Subsequent studies using urethane-induced A/J mouse lung tumor development⁴⁶ and human breast cancer⁴⁷ have shown no correlation between telomerase activity and p53 mutations although it was correlated with p53 protein accumulation.⁴⁷ It is tempting to speculate the role of p53 in cellular immortalization/senescence, and as carcinogenesis is a multistep process, p53 inactivation and telomerase activation may be independent and cooperating molecular events in certain cellular contexts.

Protein phosphorylation, a key posttranslational mode of regulating protein structure and function is also an important regulator of telomerase activity. Telomerase activity was markedly inhibited in the presence of protein phosphatase 2A (PP2A) but not of protein phosphatase 1 or protein phosphatase 2B.⁴⁸ The inhibition was also seen in the presence of a nonspecific protein phosphatase-alkaline phosphatase and prevented by the PP2A inhibitor okadaic acid. These results suggest that telomerase may exist in two different configurations that can be switched on or off by reversible phosphorylation and dephosphorylation. Potential mediators of this regulatory process are the oncogene *Akt* and all isoforms of protein kinase C that are capable of hTERT phosphorylation and subsequent enhancement of telomerase activity.⁴⁹ Treatment with inhibitor of *Akt* signaling pathway, the PI3 ki-

nase inhibitor Wortmannin, inhibits the phosphorylation of hTERT and telomerase activity.²⁸ Moreover, activation of telomerase in human CD4⁺ T cells required the phosphorylation of hTERT with subsequent translocation from the cytoplasm to the nucleus.⁵⁰ These results highlight the notion that telomerase may be a component of signaling pathways regulating numerous key processes such as apoptosis, survival, proliferation, and tumorigenesis. And, given the complex nature of telomerase regulation, it is plausible that there is cell-type-specific response to multiple environmental stimuli, including growth hormones,⁵¹ estrogen,⁵² and antigen receptor engagement.^{13,14} They also highlight the importance of determining the intracellular factors that control telomerase activity that are important issues for both cancer research and therapy.

Several issues are raised in assessing the potential significance of targeting telomerase as an anticancer drug. One, does the level of telomerase have prognostic significance? The predictive value of telomerase activity as a prognostic factor has been amply demonstrated. Telomere length and telomerase activity predict survival in patients with B-cell chronic lymphocytic leukemia.⁵³ Comparison of diagnostic and follow-up samples from acute and chronic leukemia patients revealed significantly increased telomerase activity in diagnostic specimens compared with specimens obtained after treatment initiation.⁵⁴ Early-stage neuroblastomas generally correlated with a favorable outcome, whereas the late-stage disease had high telomerase activity and correlated with a poor outcome.⁵⁵ High hTERT mRNA levels correlated with tumor recurrence in patients with Wilms' tumor.⁵⁶

Secondly, is there a relationship between telomerase activity and chemosensitivity? Recent studies suggest a complex relationship between telomerase activity and chemosensitivity. Exposure of cervical cancer cells to cisplatin resulted in rapid shortening and degradation of telomeres before the onset of cellular apoptosis⁵⁷ suggesting that telomeres are physical targets of anticancer drugs such as cisplatin. Thus, telomere loss may contribute directly to cisplatin cytotoxicity. Thirdly, because telomerase activity is essential for protecting DNA, can DNA-damaging agents increase chemosensitivity by inhibiting telomerase activity? Indirect support for this is provided by the findings of Kondo and colleagues,⁵⁸ who showed that chemoresistant subclones of malignant glioblastoma cell lines U251-MG demonstrated high telomerase activity whereas the cisplatin-sensitive U87-MG clone did not.

Given that telomerase activity is regulated at multiple levels by oncogenes, tumor suppressor genes and other stimuli involved in cell differentiation, can chemotherapeutic agents or radiation modulate telomerase activity? If so, what are the mechanisms involved? Lin and co-workers² show that malignant lymphoma cells down-regulated telomerase activity in response to a variety of chemotherapeutic agents as well as radiation. Furthermore, the decline in telomerase activity that correlated with cellular growth arrest in T-cell lymphoblastic lymphoma (ALL) cells was not observed in Raji, a Burkitt's-derived cell line. Raji cells were susceptible to radiation-

induced cytotoxicity however no down-regulation of telomerase activity was seen in these cells in contrast to T-ALLs. By comparison, chemotherapeutic agents that induced differentiation of acute myeloid leukemias resulted in inhibition of telomerase activity.^{59,60} Down-regulation of telomerase activity and hTERT was observed in acute leukemia cells as well as nonmalignant hematopoietic cells treated with interferon- γ .⁶¹ Tamoxifen, an anti-estrogenic agent used to treat breast cancer also inhibited the levels of telomerase in estrogen receptor-positive breast cancer cell lines.⁶²

What are the potential mechanisms for the down-regulation? A direct inhibition by the antitumor drugs can be excluded as testing of cell extracts to the agents failed to inhibit telomerase activity. Lin and colleagues,² raise the possibility of cell-cycle arrest-mediated telomerase modulation as demonstrated by the accumulation of p27Kip1 protein. Although many of the previous studies also observed concomitant G₂-M or G₀/G₁ phase arrest with decline with telomerase activity, modulation of telomerase activity during cell cycle remains controversial. Whereas earlier studies demonstrated increasing levels of telomerase activity with progression to the S phase and decrease during G₂-M phase,⁶³ more recent studies using flow cytometrically sorted cells indicated that telomerase activity is detected throughout the cell cycle in proliferating immortal cells,⁶⁴ with decline in activity seen when cells exit the cell cycle or become quiescent. Thus what remains unclear is the nature of the primary event; does telomerase inhibition lead to cell death or is the decline in telomerase mediated by cell cycle exit by apoptotic cells?

The expression of a mutant catalytic subunit of human telomerase in malignant cells resulted in complete inhibition of telomerase activity, telomeric shortening, cell growth arrest, and subsequent death of tumor cells.⁶⁵ Furthermore, there was variable time delay between telomerase inhibition and growth arrest that correlated with initial telomere length. This suggests that inhibition of telomerase does not have direct cytostatic effects on tumor cells.

Direct inhibition of telomerase using peptide nucleic acid and 2'-O-MeRNA oligomers resulted in progressive telomere shortening and subsequent apoptosis.⁶⁶ Interestingly, telomeric shortening was reversible in that the telomeres were able to regain their initial lengths with withdrawal of the inhibitor. In addition, tumors with shorter telomeres were most susceptible to inhibitory effect of peptide nucleic acid inhibitors and 2'-O-MeRNA oligomers. With this in mind, can down-regulation of telomerase activity enhance the response to anticancer drugs? Studies of brain tumors have shown that inhibition of telomerase by an anti-sense telomerase expression vector decreased telomerase activity and also increased susceptibility to cisplatin-induced apoptosis in the highly aggressive glioblastoma multiforme U251-MG clone.⁵⁸ Hammerhead ribozyme cleavage of telomerase mRNA *in vitro* diminished the abundance of hTERT mRNA, inhibited telomerase activity resulting in shortened telomeres, inhibition of net growth, and induction of apoptosis.⁶⁷ Furthermore, ribozyme cleavage of telomerase mRNA

increased the sensitivity of breast cancer cells to inhibitors of topoisomerase but not to a number of other cytotoxic drugs. These observations support the notion that telomerase inhibitors can be used as adjuvant therapy and that synergistic cytotoxic effects may be determined by the nature of the specific tumor.

Cell-type-specific modulation of telomerase activity has been attributed to genetic aberrations characteristic of the specific neoplasm. Moreover, different lymphoid lineages may have different rates of telomere shortening.¹⁸ This may partly explain the lineage-specific differences in telomerase down-regulation reported by Lin and colleagues.² Alternatively, although not addressed by Lin and colleagues,² the original telomere length may have contributed to the regulation of the telomerase activity and thus response to chemotherapeutic agents.

What happens to telomerase activity with acute telomere loss as seen with cisplatin treatment? Can cancer cells bypass this mechanism with a survival pathway resulting in feedback increase in telomerase hTERT as suggested by Lin and colleagues?² Recent studies using telomerase RNA null (mTerc^{-/-}) mice demonstrate that telomere dysfunction (ie, presence of chromosomal ends with no detectable telomere signals or signal-free ends, aneuploidy, and end-to-end chromosome fusions) rather than telomerase activity *per se* was the principal determinant governing chemosensitivity to agents that induced double-stranded DNA breaks.⁶⁸ Telomerase reconstitution however restored genomic integrity and chemoresistance. Can resistant clones be detected by the determining residual amount of telomerase activity as well as telomere length? Novel quantitative, sensitive, and rapid flow cytometric methods to detect telomere length⁶⁹ and real-time polymerase chain reaction techniques to detect telomerase activity⁷⁰ in clinical specimens⁷¹ may be useful in this respect.

As tumorigenesis is a process of multiple steps, more studies are needed to demonstrate whether the maintenance of telomeres in cancer cells may be involved in overcoming the cellular mechanisms controlling apoptosis/survival and senescence. The work of Lin and colleagues² suggests that telomerase-signaling pathways are differentially regulated in different cellular contexts and lineages. Determination of global changes in gene expression after telomerase inhibition by antisense oligonucleotides or ribozymes will shed light on the coordinate signaling pathways involved. Given the complexity of the signaling pathways involved in apoptosis and DNA damage, these novel technologies will be potent tools to determine the synergistic effects of cytotoxic agents.

Although the mechanisms involved in telomerase regulation are far from established, a better understanding of the regulation of telomerase activity will provide a basis for further investigation and manipulation of telomerase activity as a potential therapeutic modality. Recent work on telomerase inhibitors supports the idea that a reasonable strategy could be to use them as adjuvant therapies in combination with surgery, radiation, chemotherapy, as well as new anti-angiogenesis compounds. This highlights the need for thorough investigations into the mod-

ulation of telomerase by these agents individually and in combination.

References

- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW: Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994, 266:2011-2015
- Lin Z, Lim S, Viani MA, Sapp M, Lim MS: Down-regulation of telomerase activity in malignant lymphomas by radiation and chemotherapeutic agents. *Am J Pathol* 2001, 159:711-719
- Blackburn EH: Telomeres and their synthesis. *Science* 1990, 249:489-490
- de Lange T: Human telomeres are attached to the nuclear matrix. *EMBO J* 1992, 11:717-724
- Counter CM: The roles of telomeres and telomerase in cell life span. *Mutat Res* 1996, 366:45-63
- Harley CB, Futcher AB, Greider CW: Telomerase shorten with aging. *Nature* 1990, 345:458-460
- Feng J, Funk WD, Wang S-S, Weinrich SL, Avilion AA, Chiu C-P, Adams WH, Greider CW, Villeponteau B: The RNA component of human telomerase. *Science* 1995, 269:1236-1241
- Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB, Arruda I, Robinson MO: A mammalian telomerase-associated protein. *Science* 1997, 275:973-977
- Harrington L, Zhou W, McPhail T, Oulton R, Yeung DSK, Mar V, Bass MB, Robinson MO: Human telomerase contains evolutionary conserved catalytic and structural subunits. *Genes Dev* 1997, 11:3109-3115
- Morin GB: The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989, 53:521-529
- Counter CM, Meyerson M, Eaton EN, Ellisen LW, Caddle SD, Haber DA, Weinberg RA: Telomerase activity is restored in human cells by ectopic expression of hTERT(hEST2), the catalytic subunit of telomerase. *Oncogene* 1998, 16:1217-1222
- Nakamura TM, Morin GB, Chapman B, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR: Telomerase catalytic subunit homologs from fission yeast and humans. *Science* 1997, 277:955-959
- Igarashi H, Sakaguchi N: Telomerase activity is induced in human peripheral B lymphocytes by the stimulation to antigen receptor. *Blood* 1997, 89:1299-1307
- Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, Shay JW, Ishioka S, Yamakido M: Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol* 1995, 155:3711-3715
- Bodnar AG, Kim NW, Effros RB, Chiu C-P: Mechanism of telomerase induction during T cell activation. *Exp Cell Res* 1996, 228:58-64
- Weng N-P, Granger L, Hodes RJ: Telomere lengthening and telomerase activation during human B cell differentiation. *Proc Natl Acad Sci USA* 1997, 94:10827-10832
- Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ, Went N: Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. *Proc Natl Acad Sci USA* 1999, 96:5147-5152
- Son NH, Murray S, Yanovski J, Hodes RJ, Weng N: Lineage-specific telomere shortening and unaltered capacity for telomerase expression in human T and B lymphocytes with age. *J Immunol* 2000, 165:1191-1196
- Cong YS, Wen J, Bacchetti S: The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. *Hum Mol Genet* 1999, 8:137-142
- Horikawa I, Cable PL, Afshari C, Barrett JC: Cloning and characterization of the promoter region of human telomerase reverse transcriptase gene. *Cancer Res* 1999, 59:826-830
- Fujimoto K, Takahashi M: Telomerase activity in human leukemic cell lines is inhibited by antisense pentadecadeoxynucleotides targeted against c-myc mRNA. *Biochem Biophys Res Commun* 1997, 241:775-781
- Klingelhutz AJ, Foster SA, McDougall JK: Telomerase activation by

- the E6 gene product of human papillomavirus type 16. *Nature* 1996, 380:79–82
23. Greenberg RA, O'Hagan RC, Deng H, Xiao Q, Hann SR, Adams RR, Lichtsteiner S, Chin L, Morin GB, DePinho RA: Telomerase reverse transcriptase gene is a direct target of c-Myc but is not functionally equivalent in cellular transformation. *Oncogene* 1999, 18:1219–1226
24. Burger AM, Fiebig HH, Kuettel MR, Lautenberger JA, Kung HF, Rhim JS: Effect of oncogene expression on telomerase activation and telomere length in human endothelial, fibroblast and prostate epithelial cells. *Int J Oncol* 1998, 13:1043–1048
25. Yu CC, Lo SC, Wang TC: Telomerase is regulated by protein kinase C-zeta in human nasopharyngeal cancer cells. *Biochem J* 2001, 355:459–464
26. Mandal M, Kumar R: Bcl-2 modulates telomerase activity. *J Biol Chem* 1997, 272:14183–14187
27. Kharbanda S, Kumar V, Dhar S, Pandey P, Chen C, Majumder P, Yuan ZM, Whang Y, Strauss W, Pandita TK, Weaver D, Kufe D: Regulation of the hTERT telomerase catalytic subunit by the c-Abl tyrosine kinase. *Curr Biol* 2000, 10:568–575
28. Kang SS KT, Kwon DY, Do SI: Akt protein kinase enhances human telomerase activity through phosphorylation of telomerase reverse transcriptase subunit. *J Biol Chem* 1999, 274:13085–13090
29. Xu H-J, Shou Y, Ji W, Pereng G-S, Kruzelock R, Kong C-T, Bast RC, Mills GB, Li J, Hu S-X: Reexpression of the retinoblastoma protein in tumor cells induces senescence and telomerase inhibition. *Oncogene* 1997, 15:2589–2596
30. Yang H, Kyo S, Takatura M, Sun L: Autocrine transforming growth factor beta suppresses telomerase activity and transcription of human telomerase reverse transcriptase in human cancer cells. *Cell Growth Differ* 2001, 12:119–127
31. Terasaki M, Kato S, Sugita Y, Shigemori M, Morimatsu M: Adenovirus-mediated over-expression of p21Waf-1 differentially affects the proliferation, telomerase activity, and expression of glial fibrillary acidic protein in human glioma cells. *Int J Oncol* 1999, 14:63–69
32. Horikawa I, Oshimura M, Barrett JC: Repression of the telomerase catalytic subunit by a gene on human chromosome 3 that induces cellular senescence. *Mol Carcinog* 1998, 22:761–766
33. Cuthbert AP, Bond J, Trott DA, Gill S, Broni J, Marriot A, Khoudoli G, Parkinson EK, Cooper CS, Newbold RF: Telomerase repressor sequences on chromosome 3 and induction of permanent growth-arrest in human breast cancer cells. *J Natl Cancer Inst* 1999, 91:37–45
34. Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR: Tissue-specific alternate splicing of human telomerase reverse transcriptase (hTERT) influences telomere lengths during human development. *Int J Cancer* 2001, 91:644–649
35. Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR: Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. *Cancer Res* 1998, 58:4168–4172
36. Zhang A, Zheng C, Lindvall C, Hou M, Ekedahl J, Lewensohn R, Yan Z, Yang X, Henriksson M, Blennow E, Nordenskjold M, Zetterberg A, Bjorkholm M, Gruber A, Xu D: Frequent amplification of the telomerase reverse transcriptase gene in human tumors. *Cancer Res* 2000, 60:6230–6235
37. Dessain SK, Yu H, Reddel RR, Beijersbergen RL, Weinberg RA: Methylation of the human telomerase gene CpG island. *Cancer Res* 2000, 60:537–541
38. Devereux TR, Horikawa I, Anna CH, Annab LA, Afshari CA, Barrett JC: DNA methylation analysis of the promoter region of the human telomerase reverse transcriptase (hTERT) gene. *Cancer Res* 1999, 59:6087–6090
39. Nakamura M, Saito H, Ebinuma H, Wakabayashi K, Saito Y, Takagi T, Nakamoto N, Ishii H: Reduction of telomerase activity in human liver cancer cells by a histone deacetylase inhibitor. *J Cell Physiol* 2001, 187:392–401
40. Ferrara FF, Fazi F, Bianchini A, Padula F, Gelmetti V, Minucci S, Mancini M, Pelicci PG, Lo Coco F, Nervi C: Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia. *Cancer Res* 2001, 61:2–7
41. Bernhard D, Loffler M, Hartmann BL, Yoshida M, Kofler R, Csordas A: Interaction between dexamethasone and butyrate in apoptosis induction: non-additive in thymocytes and synergistic in a T cell-derived leukemia cell line. *Cell Death Differ* 1999, 6:609–617
42. Holt SE, Wright WE, Shay JW: Regulation of telomerase activity in immortal cell lines. *Mol Cell Biol* 1996, 16:2932–2939
43. Li H, Cao Y, Berndt MC, Funder JW, Liu J-P: Molecular interactions between telomerase and the tumor suppressor protein p53 in vitro. *Oncogene* 1999, 18:6785–6794
44. Ueda M, Ouhtit A, Bito T, Nakazawa K, Lubbe J, Ichihashi M, Yamasaki H, Nakazawa H: Evidence for UV-associated activation of telomerase in human skin. *Cancer Res* 1997, 57:370–377
45. Gollahon LS, Shay JW: immortalization of human mammary epithelial cells transfected with mutant p53 (273his). *Oncogene* 1996, 12:715–725
46. Ohno J, Horio Y, Sekido Y, Hasegawa Y, Takahashi M, Nishizawa J, Saito H, Ishikawa F, Shimokata K: Telomerase activation and p53 mutations in urethane-induced A/J mouse lung tumor development. *Carcinogenesis* 2001, 22:751–756
47. Roos G, Nilsson P, Cajander S, Nielsen NH, Arnerlov C, Landberg G: Telomerase activity in relation to p53 status and clinico-pathological parameters in breast cancer. *Int J Cancer* 1998, 79:343–348
48. Li H, Zhao LL, Funder JW, Liu JP: Protein phosphatase 2A inhibits nuclear telomerase activity in human breast cancer cells. *J Biol Chem* 1997, 272:16729–16732
49. Li H, Zhao L, Yang Z, Funder JW, Liu JP: Telomerase is controlled by protein kinase C alpha in human breast cancer cells. *J Biol Chem* 1998, 273:33436–33442
50. Liu K, Hodes RJ, Weng NP: Telomerase activation in human T lymphocytes does not require increase in telomerase reverse transcriptase (hTERT) protein but is associated with hTERT phosphorylation and nuclear translocation. *J Immunol* 2001, 166:4826–4830
51. Kiaris H, Schally AV: Decrease in telomerase activity in U-87MG human glioblastomas after treatment with an antagonist of growth hormone-releasing hormone. *Proc Natl Acad Sci USA* 1999, 96:226–231
52. Misiti S, Nanni S, Fontemaggi G, Cong YS, Wen J, Hirte HW, Piaggio G, Sacchi A, Pontecorvi A, Bacchetti S, Farsetti A: Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells. *Mol Cell Biol* 2000, 20:3764–3771
53. Bechter OE, Eisterer W, Pall G, Hilbe W, Kuhr T, Thaler J: Telomere length and telomerase activity predict survival in patients with B cell chronic lymphocytic leukemia. *Cancer Res* 1998, 58:4918–4922
54. Engelhardt M, Mackenzie K, Drullinsky P, Silver RT, Moore MA: Telomerase activity and telomere length in acute and chronic leukemia, pre- and post-ex vivo culture. *Cancer Res* 2000, 60:610–617
55. Streutker CJ, Thorner P, Fabricius N, Weitzman S, Zielenska M: Telomerase activity as a prognostic factor in neuroblastomas. *Pediatr Dev Pathol* 2001, 4:62–67
56. Dome JS, Chung S, Bergemann T, Umbricht CB, Saji M, Carey LA, Grundy PE, Perlman EJ, Breslow NE, Sukumar S: High telomerase reverse transcriptase (hTERT) messenger RNA level correlates with tumor recurrence in patients with favorable histology Wilms' tumor. *Cancer Res* 1999, 59:4301–4307
57. Ishibashi T, Lippard SJ: Telomere loss in cells treated with cisplatin. *Proc Natl Acad Sci USA* 1998, 95:4219–4223
58. Kondo Y, Kondo S, Tanaka Y, Haqqi T, Barna BP, Cowell JK: Inhibition of telomerase increases the susceptibility of human malignant glioblastoma cells to cisplatin-induced apoptosis. *Oncogene* 1998, 16:2243–2248
59. Xu D, Gruber A, Peterson C, Pisa P: Suppression of telomerase activity in HL60 cells after treatment with differentiating agents. *Leukemia* 1996, 10:1354–1357
60. Zhang W, Piatyszek MA, Kobayashi T, Estey E, Andreeff M, Deisseroth AB, Wright WE, Shay JW: Telomerase activity in human acute myelogenous leukemia: inhibition of telomerase activity by differentiation-inducing agents. *Clin Cancer Res* 1996, 2:799–803
61. Xu D, Erickson S, Szeps M, Gruber A, Sangfelt O, Einhorn S, Pisa P, Grander D: Interferon alpha down-regulates telomerase reverse transcriptase and telomerase activity in human malignant and nonmalignant hematopoietic cells. *Blood* 2000, 96:4313–4318
62. Aldous WK, Marean AJ, DeHart MJ, Matej LA, Moore KH: Effects of tamoxifen on telomerase activity in breast carcinoma cell lines. *Cancer* 1999, 7:1523–1529
63. Zhu X, Kumar R, Mandal M, Sharma N, Sharma HW, Dhingra U, Sokolowski JA, Hsiao R, Narayanan R: Cell cycle-dependent modulation of telomerase activity in tumor cells. *Proc Natl Acad Sci USA* 1996, 93:6091–6095

64. Holt SE, Aisner DL, Shay JW, Wright WE: Lack of cell cycle regulation of telomerase activity in human cells. *Proc Natl Acad Sci USA* 1997, 94:10687–10692
65. Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JH, Meyerson M, Weinberg RA: Inhibition of telomerase limits the growth of human cancer cells. *Nat Med* 1999, 5:1164–1170
66. Herbert B, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW, Corey DR: Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *Proc Natl Acad Sci USA* 1999, 96:14276–14281
67. Ludwig A, Saretzki G, Holm PS, Tiemann F, Lorenz M, Emrich T, Harley CB, von Zglinicki T: Ribozyme cleavage of telomerase mRNA sensitizes breast epithelial cells to inhibitors of topoisomerase. *Cancer Res* 2001, 61:3053–3061
68. Lee KH, Rudolph KL, Ju YJ, Greenberg RA, Cannizzaro L, Chin L, Weiler SR, DePinho RA: Telomere dysfunction alters the chemotherapeutic profile of transformed cells. *Proc Natl Acad Sci USA* 2001, 98:3381–3386
69. Brummendorf TH, Holyoake TL, Rufer N, Barnett MJ, Schulzer M, Eaves CJ, Eaves AC, Lansdorp PM: Prognostic implications of differences in telomere length between normal and malignant cells from patients with chronic myeloid leukemia measured by flow cytometry. *Blood* 2000, 95:1883–1890
70. Kim NW, Wu F: Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Res* 1997, 25:2595–2597
71. Dasi F, Lledo S, Garcia-Granero E, Ripoll R, Marugan M, Tormo M, Garcia-Conde J, Alino SF: Real-time quantification in plasma of human telomerase reverse transcriptase (hTERT) mRNA: a simple blood test to monitor disease in cancer patients. *Lab Invest* 2001, 81:767–769