

Telomeres and Telomerase in Lung Cancer

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Abstract: Protected telomeres ensure normal chromosomal segregation during mitosis but at the same time can endow genetically abnormal cancer cells with immortality. Telomerase has a pivotal role in telomere protection, both in normal and cancer cells. Understanding the functional interplay between telomere shortening and telomerase expression in cancer cells is of critical importance to elucidating the precise mechanisms by which these cells are able to bypass telomere crisis and become immortal.

Key Words: Cancer, Lung, Telomere, Telomerase.

(*J Thorac Oncol.* 2008;3: 1085–1088)

TELOMERES: AN ANTICANCER BARRIER

Telomeres are the ends of linear genomes. Made of multiple copies of a TTAGGG sequence, telomeres protect chromosomes from degradation, irregular recombination and end-to-end fusions.¹ Normally, a sheltering complex—*shelterin*—composed of at least six telomere associated proteins, caps the telomeres, protecting them from being recognized as double strand breaks by the DNA damage response (DDR).² Telomeres shorten with every cell division. Somatic primary cells in cell culture shorten their telomeres by 50 to 200 base pairs after each round of replication until they reach a critical length below which the shelterin complex becomes inefficient. This leaves the telomeres “un-capped,” which in turn activates the DDR and triggers cellular senescence.^{1,2} This process is known as the “mitotic clock for aging.”³ The current hypothesis of telomere involvement in cancer (Figure 1A) states that proliferative preneoplastic cells suffer persistent telomere shortening leading to massive senescence (mortality stage I) in all but a few positively selected cells, which are able to bypass senescence by altering their DDR by mutation or silencing of DDR-related proteins such as ataxia telangiectasia mutated, p53, and p16.⁴ These cells extend

their life span and continue losing telomere fragments until their telomeres become dysfunctional, causing genomic instability and subsequent apoptosis (mortality stage 2). According to this model, during this process a small subpopulation of cells is able to avoid apoptosis by maintaining their telomere length. Telomere length maintenance at this stage is related to the expression of telomerase, which adds TTAGGG fragments to the end of the telomeres, lengthening and maintaining them in the capped conformation. More rarely, telomere elongation can also be achieved through homologous recombination between telomeric sequences: the alternative lengthening mechanism. Thus, according to the current model, the progeny of the few, genetically unstable, immortal cells that escape both mortality stages may allow the progression of a preneoplastic lesion towards malignant stages.^{1,3–6}

Telomerase is a large DNA polymerase ribonucleoprotein (RNP) complex containing an RNA subunit (telomerase RNA component [TERC]) and a protein component (telomerase reverse transcriptase [TERT]). Most somatic cells do not show detectable telomerase activity mainly because of lack of telomerase expression. However, stem and embryonic cells do express telomerase as a mechanism to prevent telomere attrition.⁷

TELOMERE MAINTENANCE AND TELOMERASE REGULATION

Because of its importance for cell fate, telomere length is finely regulated. Telomerase activity is the main mechanism for telomere maintenance and thus, telomerase activity itself is also carefully controlled (Figure 1B).

The active telomerase RNP consists of three subunits: the telomerase reverse transcriptase (TERT), the TERC, and dyskerin 1.⁸ The catalytic activity of this enzyme resides in the TERT component, and thus the regulation of TERT mRNA expression seems to be the most important step for telomerase activation. In this context, many oncogenic and tumor suppressor pathways have been shown to regulate TERT mRNA transcription. C-myc, RAS, E6, STAT 3, and estrogens are activators of TERT, whereas Mad 1, p53, TGF- β , RAK, BRIT1, and MDM2 are inhibitors. TERT transcription is also regulated by epigenetic modifications of its promoter, which contains clusters of CpG islands which can be methylated, and thus silenced, by DNA methyl transferases. Besides transcriptional regulation, TERT activity may be regulated by alternative splicing and posttranslational modifications.⁷ Active telomerase RNP requires that transiently expressed TERT assembles with constitutively accu-

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Disclosure: The authors declare no conflicts of interest.

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ISSN: 1556-0864/08/0310-1085

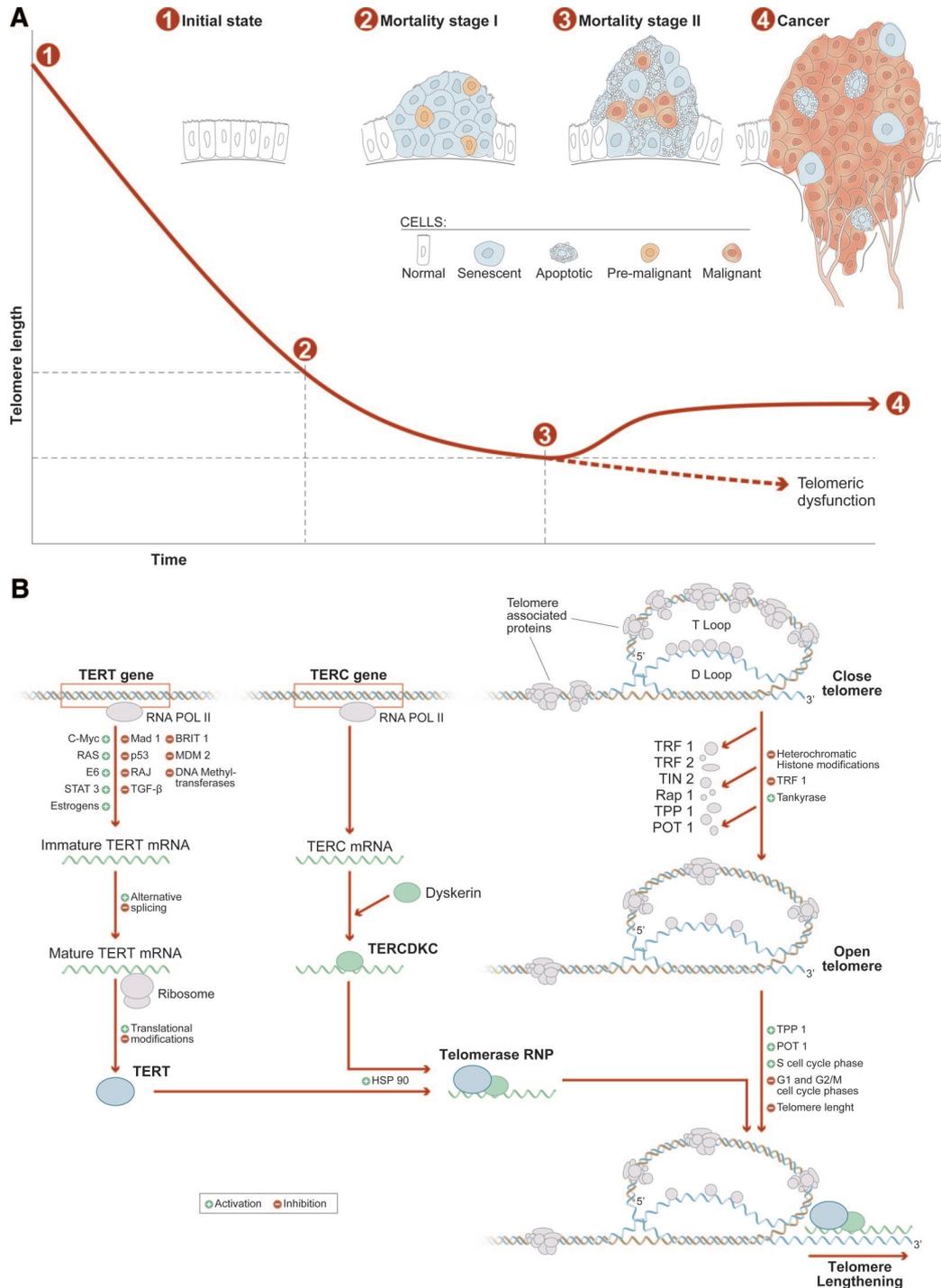


FIGURE 1. A, Theoretical model of the association between telomere length and the carcinogenesis process. The cells continuously proliferating in preneoplastic lesion suffer persistent telomere shortening leading to senescence (mortality stage I). Some cells are able to bypass senescence by altering their DDR and keep proliferating. These cells extend their lifespan until their telomeres become dysfunctional, entering apoptosis (mortality stage II). A small subpopulation of cells with genetic abnormalities escapes both mortality stages and progress towards malignancy. B, Summary of the telomerase regulation and telomere elongation pathway. Abbreviations: telomerase reverse transcriptase (TERT), telomerase RNA component (TERC), RNA polymerase II (RNA POL II), dyskerin (DKC), telomerase ribonucleoprotein (telomerase RNP), telomeric repeat-binding factor (TRFs) 1 and 2, TRF1-interacting nuclear factor 2 (TIN2), TRF2-interacting protein 1 (Rap1), adrenocortical dysplasia protein homolog (TPP1), and protection of telomeres protein 1 (POT1).

mulated TERC-dyskerin complexes. This assembling process requires the presence of HSP90. Recent studies show that alterations in TERC processing can limit telomerase activity.⁹

Besides telomerase RNP regulation, telomere maintenance depends on telomere structure as well. At least two factors have been shown to regulate telomere structure: telomere chromatin modifications and alterations of the telomere-binding protein complex. Both factors determine whether the telomeres are in a “closed” or “open” conformation to allow telomerase RNP-based elongation.^{2,3,7} The shelterin protein complex is formed by six telomere-specific binding factors: telomeric repeat-binding factors (TRFs) 1 and 2, TRF1-interacting nuclear factor 2 (TIN2), TRF2-interacting protein 1 (Rap1), adrenocortical dysplasia protein homolog (TPP1) and protection of telomeres protein 1 (POT1). They are in charge of maintaining the telomere “capped,” making it invisible to the DDR. Alterations in the shelterin proteins cause telomere “un-capping” and cell senescence.^{2,7} The telomere open status is also driven by the enzyme Tankyrase.¹⁰

Finally, telomerase ability to maintain telomere length also depends on the accessibility of the active telomerase RNP to each telomere that requires elongation. Telomerase RNP does not elongate all telomeres at the same time and it seems to act preferentially on the shortest telomeres of the cell during the S phase of the cycle.^{11,12}

TELOMERES AND TELOMERASE REGULATION IN LUNG CANCER

Telomerase is expressed in most human cancers, including lung cancers. As in many other cancer types, lung cancer cells avoid the progressive attrition of telomeres by expressing telomerase. Using a polymerase chain reaction-based telomeric repeat amplification protocol assay, early studies reported finding telomerase activity in most primary lung cancer samples.^{13,14} Furthermore, several studies using animal models and human non-small cell lung cancer (NSCLC) tissues have reported that TERT mRNA and TERT protein are overexpressed in lung cancer biopsies compared with normal lung tissues.^{4,15}

In recent years, the oncogenic nature of telomerase has been a debatable issue. In an exhaustive review, Harley¹⁶ provided strong arguments against this concept. Supporting that line of evidence, recent experimental data show that reintroduction of telomerase in low telomerase expressing somatic cells does not transform them. Ramirez et al.¹⁷ overexpressed cyclin-dependent kinase 4 and hTERT in human bronchial epithelial cells, resulting in immortal cell lines, pointing to the necessity of bypassing senescence before cell immortalization with telomerase. Cyclin-dependent kinase 4 overexpression prevented p16INK4a-associated senescence induced by the *in vitro* culture conditions (mortality stage I), whereas expression of hTERT bypassed telomere dysfunction (mortality stage II). The created cell lines provide a useful model that can be genetically manipulated to identify genetic and epigenetic differences between tumor and normal cells. In fact, using these immortalized human bronchial epithelial cells, the same group has more recently reported that p53 knockdown, K-RASV12, and mutant epidermal growth factor

receptor contribute to lung cancer tumorigenesis, but are not enough to achieve full malignant transformation and *in vivo* tumor formation.¹⁸

Recently, a mutated version of the mouse telomerase catalytic subunit (mTerc) has been incorporated to the well-characterized K-rasG12D mouse lung cancer model. K-ras(G12D) mTerc(−/−) mice with telomere dysfunction but intact p53 exhibited increased lung epithelial apoptosis, delayed tumor formation, and increased life span relative to K-ras(G12D) mTerc(+/−) mice with intact telomere function.¹⁹

Lantuejoul et al.⁴ proposed that telomere length diminishes throughout the multistep human lung carcinogenesis progression until a point when a stable short length is reached and telomerase is activated. Telomere length is decreased in atypical adenomatous hyperplasias and bronchioalveolar carcinomas concomitant with positive expression of hTERT mRNA, indicating telomere dysfunction in the earliest phase of pulmonary carcinogenesis.²⁰ Several studies have been published on the expression of telomerase in preneoplastic lesions in lung cancer and in other tumors. In general, these reports show an increase in telomerase expression at the late dysplastic lesions. These immunocytochemical studies have been carried out by means of commercially available antibodies, the specificity of which has been recently challenged.^{4,21} Validation studies with new highly specific antibodies are needed to confirm the reported data on expression of telomerase and other related proteins.

Mechanisms for telomerase activation in lung cancer cells are not known yet. Comparative genomic hybridization studies in samples from early stages of NSCLC have shown that the genomic region that harbors hTERT gene, 5p15.33, is frequently amplified when compared with normal tissues.²² Mutations in TERT or TERC that result in telomere shortening confer increase in susceptibility to adult-onset idiopathic pulmonary fibrosis.²³ However, none of these mutations have been associated to lung cancer. The expression of other telomere-associated proteins has been also reported as altered in lung cancer. Quantitative polymerase chain reaction studies showed that TRF1 expression is lower in NSCLC tissues when compared with the adjacent normal tissues.²⁴

TELOMERES AND ANTICANCER THERAPIES

The differences between normal and cancer cells in terms of telomerase expression and telomere length provide a therapeutic opportunity for telomerase inhibition-based therapies. Cancer patients are less likely to develop resistance to telomerase-based therapies than to other cancer drugs, and telomerase-based therapies are unlikely to cause tissue toxicity to normal nontelomerase expressing cells.⁶

There are two different approaches for telomerase-based therapies that have already been used in clinical trials: direct enzyme inhibition and active telomerase immunotherapy. Different compounds with telomerase activity inhibition properties have been studied *in vitro* and in animal models. Three of them, BIBR1532, GRN163, and GRN163L have been proven to have anticancer properties in several animal models.²⁵ Other small-molecule modulators of telomerase/

telomere biology, notably G-quadruplex ligands such as RHPS4, are in preclinical development.²⁶ GRN163L is the only one being tested in humans. Four trials with this oligonucleotide-based compound targeting the telomerase RNA template are in phase I for different cancer types. Specifically, study NCT00510445 was designed to determine the safety and the maximum tolerated dose of GRN163L when administered in combination with a standard paclitaxel/carboplatin regimen to patients with advanced or metastatic NSCLC. This lung cancer trial is the first to study GRN163L in combination with standard chemotherapy. Recruitment for this study is due to be completed in December 2008.⁶

Cancer immunotherapies use self antigens up-regulated in cancer cells, to overcome self-recognition by normal cells. Currently, there are some products which use synthetic TERT peptides to induce antigen-presenting cells to produce an immune response against cancer cells expressing TERT antigens. The most advanced ones are GV1001 and GRNVAC1. Trial NCT00509457 was designed to examine the safety and efficacy of GV1001 telomerase peptide vaccination in patients with NSCLC after having been treated with conventional therapy with radiotherapy and docetaxel as a radiosensitizer. The trial started in November 2006 and is still recruiting patients.⁶ The vaccine Vx-001 using a hTERT cryptic peptide has completed a “proof of principle” Phase I/II clinical trial presented at ASCO 2008. Vx-001 has also obtained an orphan drug designation for NSCLC from EMEA.²⁷

The results of the mentioned clinical trials, and new trials designed to test new antitelomerase drugs need to be followed carefully as they may provide new avenues for isolated or combined targeted therapies for lung cancer. It could be of interest to see if there is a gene signature linked to the molecular status of telomere-related proteins that could predict the response to telomerase inhibitors. The question about the relevance of telomeres and telomerase pathway for lung cancer therapy will hopefully be solved in the very next future.

ACKNOWLEDGMENTS

Supported by the “UTE project CIMA,” the Spanish Ministry of Science and Education, the Spanish Fondo de Investigaciones Sanitarias (RTICC) the Government of Navarra, the EU Marie Curie Program, and a Ramón y Cajal Fellowship.

The authors thank Drs. Rafael Rosell and Ruben Pio for critical reading of the manuscript and Mr. Heber Longas for the artwork included in this review.

REFERENCES

- Artandi SE, Attardi LD. Pathways connecting telomeres and p53 in senescence, apoptosis, and cancer. *Biochem Biophys Res Commun* 2005; 331:881–890.
- de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 2005;19:2100–2110.
- Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet* 2005;6:611–622.
- Lantuejoul S, Salon C, Soria JC, Brambilla E. Telomerase expression in lung preneoplasia and neoplasia. *Int J Cancer* 2007;120:1835–1841.
- Blasco MA. Mice with bad ends: mouse models for the study of telomeres and telomerase in cancer and aging. *Embo J* 2005;24:1095–1103.
- Harley CB. Telomerase and cancer therapeutics. *Nat Rev Cancer* 2008; 8:167–179.
- Flores I, Benetti R, Blasco MA. Telomerase regulation and stem cell behaviour. *Curr Opin Cell Biol* 2006;18:254–260.
- Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR. Protein composition of catalytically active human telomerase from immortal cells. *Science* 2007;315:1850–1853.
- Collins K. Physiological assembly and activity of human telomerase complexes. *Mech Ageing Dev* 2008;129:91–98.
- Seimiya H, Muramatsu Y, Ohishi T, Tsuruo T. Tankyrase 1 as a target for telomere-directed molecular cancer therapeutics. *Cancer Cell* 2005; 7:25–37.
- Tomlinson RL, Ziegler TD, Supakorndej T, Terns RM, Terns MP. Cell cycle-regulated trafficking of human telomerase to telomeres. *Mol Biol Cell* 2006;17:955–965.
- Jady BE, Richard P, Bertrand E, Kiss T. Cell cycle-dependent recruitment of telomerase RNA and Cajal bodies to human telomeres. *Mol Biol Cell* 2006;17:944–954.
- Hiyama K, Hiyama E, Ishioka S, et al. Telomerase activity in small-cell and non-small-cell lung cancers. *J Natl Cancer Inst* 1995;87:895–902.
- Holt SE, Shay JW. Role of telomerase in cellular proliferation and cancer. *J Cell Physiol* 1999;180:10–18.
- Blanco D, Vicent S, Fraga MF, et al. Molecular analysis of a multistep lung cancer model induced by chronic inflammation reveals epigenetic regulation of p16 and activation of the DNA damage response pathway. *Neoplasia* 2007;9:840–852.
- Harley CB. Telomerase is not an oncogene. *Oncogene* 2002;21:494–502.
- Ramirez RD, Sheridan S, Girard L, et al. Immortalization of human bronchial epithelial cells in the absence of viral oncoproteins. *Cancer Res* 2004;64:9027–9034.
- Sato M, Vaughan MB, Girard L, et al. Multiple oncogenic changes (K-RAS(V12), p53 knockdown, mutant EGFRs, p16 bypass, telomerase) are not sufficient to confer a full malignant phenotype on human bronchial epithelial cells. *Cancer Res* 2006;66:2116–2128.
- Perera SA, Maser RS, Xia H, et al. Telomere dysfunction promotes genome instability and metastatic potential in a K-ras p53 mouse model of lung cancer. *Carcinogenesis* 2008;29:747–753.
- Kawai T, Hiroi S, Nakanishi K, Meeker AK. Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. *Am J Clin Pathol* 2007;127: 1–9.
- Wu YL, Dudognon C, Nguyen E, et al. Immunodetection of human telomerase reverse-transcriptase (hTERT) re-appraised: nucleolin and telomerase cross paths. *J Cell Sci* 2006;119:2797–2806.
- Kang JU, Koo SH, Kwon KC, Park JW, Kim JM. Gain at chromosomal region 5p15.33, containing TERT, is the most frequent genetic event in early stages of non-small cell lung cancer. *Cancer Genet Cytogenet* 2008;182:1–11.
- Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci USA* 2007;104:7552–7557.
- Lin X, Gu J, Lu C, Spitz MR, Wu X. Expression of telomere-associated genes as prognostic markers for overall survival in patients with non-small cell lung cancer. *Clin Cancer Res* 2006;12:5720–5725.
- Dikmen ZG, Gellert GC, Jackson S, et al. In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. *Cancer Res* 2005;65:7866–7873.
- Kelland L. Targeting the limitless replicative potential of cancer: the telomerase/telomere pathway. *Clin Cancer Res* 2007;13:4960–4963.
- Kotsakis A, Vetsika K, Christou N, et al. Clinical and immunologic response of patients with advanced solid tumors vaccinated with an optimized cryptic hTERT peptide (Vx-001). (2008 ASCO Annual Meeting) *J Clin Oncol* 2008;26 (May 20 suppl; abstract 3030).