Telomere dysfunction promotes genome instability and metastatic potential in a K-ras p53 mouse model of lung cancer

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Current mouse models of lung cancer recapitulate signature genetic lesions and some phenotypic features of human lung cancer. However, because mice have long telomeres, models to date do not recapitulate the aspects of lung carcinogenesis—telomere attrition and the genomic instability that ensues—believed to serve as key mechanisms driving lung tumor initiation and progression. To explore the contributions of telomere dysfunction to lung cancer progression, we combined a telomerase catalytic subunit (mTerc) mutation with the well-characterized K-rasG12D mouse lung cancer model. K-rasG12D mTerc−/− mice with telomere dysfunction but intact p53 exhibited increased lung epithelial apoptosis, delayed tumor formation and increased life span relative to K-rasG12D mTerc+/− mice with intact telomere function. This demonstrates that by itself, telomere dysfunction acts in a tumor-suppressive mechanism. Introduction of a heterozygous p53 mutation exerted a marked histopathological, biological and genomic impact. K-rasG12D mTerc−/− p53+/− mice developed aggressive tumors with more chromosomal instabilities and high metastatic potential, leading to decreased overall survival. Thus, we have generated a murine model that more faithfully recapitulates key aspects of the human disease. Furthermore, these findings clearly demonstrate (in an in vivo model system) the dual nature of telomere shortening as both a tumor-suppressive and tumor-promoting mechanism in lung cancer, dependent on p53 status.

Introduction

Lung cancer is the leading cause of cancer deaths in the world. Despite recent advances in treatment, the 1 year survival rate has only marginally increased from 37% in 1975 to 42% in 2000. Furthermore, the 5 years relative survival for all stages of lung cancer remains a dismal 16%, compared with 89% in breast cancer (1). Non-small cell lung cancer is typified by widespread chromosomal structural aberrations with numerous regional amplifications and deletions presumed to harbor cancer-relevant gene alterations. The mechanisms driving widespread genome instability in lung cancer are an area of active investigation (2–4). Tobacco smoke, the main causal agent in lung tumorigenesis, has been shown to cause oxidative stress leading to an increased mutational rate. In addition, tobacco smoke has recently been implicated as the direct cause of genomic instability in vitro. However, it remains unclear how tobacco smoke can cause the widespread chromosomal aberrations seen in most human lung cancer cells.

Telomeres distinguish the ends of linear chromosomes from abnormal DNA double strand breaks. In most replicating eukaryotic cells, telomeres are synthesized and maintained by the specialized enzyme, telomerase, comprising reverse transcriptase and RNA template components. Insufficient telomerase results in progressive telomere erosion in highly proliferative cells of renewing organ systems or in extended passages (5,6) in cell culture (7–9). In human cells, shortened telomeres can trigger cellular senescence responses that are dependent upon p16INK4A and p53 tumor suppressors. Loss of p16INK4A RB and/or p53 pathway function in primary human cells allows cells to bypass this senescence checkpoint and permits additional cell divisions. Under such circumstances of extended cell divisions, progressive telomere erosion ultimately leads to loss of telomere capping function, resulting in increasing chromosomal instability. This leads to progressive loss of cell viability and proliferative capacity across the cell population, ultimately resulting in ‘cellular crisis’. The cellular phenotypes of massive cell death and growth arrest are probably the by-products of rampant chromosomal instability and associated loss of essential genetic material (5,6). Emergence from crisis is a rare event in human cell culture and requires restoration of telomere function via either upregulation of telomerase activity or activation of the alternative lengthening of telomeres mechanism. The restoration of functional telomeres serves to quell DNA damage signaling and high levels of chromosomal instability, thereby enhancing the viability of cells with procancer genotypes. Thus, telomere-based crisis can either promote or suppress cancer depending on cell type and status of p53 checkpoint (see below).

In human lung tumorigenesis, the patterns of telomere attrition and telomerase activation have implicated telomere dynamics in the genesis and progression of this malignancy. In early neoplastic lesions, low levels of telomerase activity and shortened telomeres have been proposed as a potential mechanism driving cancer-promoting genomic instability. In more advanced human lung cancers, the robust and frequent activation of telomerase has suggested an enabling role for telomere maintenance in malignant progression, observations consistent with cell culture models indicating a role for telomerase in the sustained growth of primary cells (10) and in the transformation of such cells (11).

The importance of telomere dysfunction and telomerase activation has been genetically dissected in mouse model systems. Mouse genetic studies have shown that the impact of telomere dysfunction on immortalization and tumorigenesis depends upon the status of the p53 telomere checkpoint. In the presence of functional p53, telomere dysfunction serves as a barrier to tumorigenesis through the elimination of cells via senescence and apoptotic processes. With impaired p53 function, the loss of telomere function drives cancer-relevant genomic alterations (12,13) and promotes malignant transformation, most notably transformation of epithelial cell types (14). Thus, telomere dysfunction promotes chromosomal instability that drives early carcinogenesis and provides a mutator mechanism, but continual telomere erosion and dysfunction in cells with an intact p53 checkpoint response prevents further malignant progression. Nascent neoplastic murine cells experiencing crisis with intact p53 require adaptive mechanisms that include activation of alternative lengthening of telomeres and/or elimination of p53 to emerge from crisis.

The interaction between telomere dysfunction and checkpoint status in the setting of lung epithelial carcinogenesis remains undefined. This is of particular relevance to human lung cancer in which tobacco smoke exposure is the main etiological instigator of this disease.

Abbreviations: AdenoCre, adenovirus-expressing Cre recombinase; K-rasG12D, Lox-Stop-Lox K-rasG12D, PCR, polymerase chain reaction.
As noted above, tobacco smoke promotes chronic inflammation and associated telomere attrition in lung epithelial cells and directly mutates oncogenes and tumor suppressor genes, enabling cells to reach a critical threshold of cancer-promoting changes to effect malignant transformation (15,16).

In this study, we sought to explore a relevant genetic model to explore the interaction of these signaling pathways, as well as a model that recapitulates the genomic complexities seen in human lung cancer to observe the evolution of lung tumors in an in vivo setting. We focused on the impact of telomere dysfunction on the pathogenesis of non-small cell lung cancer driven by K-ras activation and p53 loss—the most common genetic alterations in the human disease. We modeled K-rasG12D-driven lung tumorigenesis in the presence and absence of telomere dysfunction and p53 activity by breeding a conditionally activatable \textit{Lox-Stop-Lox K-rasG12D} (hereafter \textit{K-rasG12D}) allele (17) with telomerase RNA component (tERC) and p53 mutant mice. The defined progression of lung tumors in the \textit{K-rasG12D} model—from an atypical adenomatous hyperplasia, adenoma and adenocarcinoma sequence—enabled us to assign a role for telomere dysfunction in driving genomic instability and promoting the metastatic potential of K-ras-initiated neoplasms.

Materials and methods

\textbf{Mating scheme and treatment}

The \textit{K-rasG12D} knockin strain with \textit{Lox-STOP-Lox} was generously provided by Tyler Jacks and David Tuveson (Massachusetts Institute of Technology, (17)). These mice were crossed with \textit{G4 mTerc}+/−, p53+/− (or p53−/−) mice to generate \textit{G5i mTerc}−/− \textit{K-rasG12D} p53+/− (or p53−/−) and \textit{G6i mTerc}+/+ \textit{K-rasG12D} p53+/− (or p53+/+) mice (supplementary Figure 1, available at \textit{Carcinogenesis} Online). Mice were genotyped using polymerase chain reaction (PCR) (conditions available on request) and infected with adenovirus-expressing Cre recombinase (AdenoCre) at 4–6 weeks of age as described previously (17). Briefly, mice were anesthetized with 2.5% 2,2,2-trichloroethanol (Sigma, St. Louis, MO) and 125 μl of AdenoCre (University of Iowa) and CaPico precipitate was administered intranasally in two instillations.

\textbf{Histological and immunohistochemical analysis}

Mice were killed at indicated time points and tissues were fixed in 10% formalin and embedded in paraffin. Lungs were inflated with formalin at a 25 psi for paraffin embedding. Apoptosis was measured by Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assay performed on 4 μM paraffin sections using Apo-Tag Peroxidase kit (S7100; Chemicon/ Millipore, Billerica, MA) according to protocol. Proliferation indices were measured from Ki-67 (Vector)-stained paraffin sections. In both proliferation and apoptosis assays, the counts represent the values of positive stained cells in five × 400 tumor fields and summed up for graphing. Polyclonal rabbit anti-p53 antibody (CMS; Vector, Burlingame, CA) was used for detection of p53 expression and pan-cytokeratin antibody (MNF116 at 1:700; DAKO, Carpinteria, CA) was used for labeling epithelial cells. For p53 expression quantification, tumors >3 mm in diameter from both \textit{G5i} and \textit{G6i} 24- to 30-week-old \textit{K-rasG12D} p53+/− mice were given a count of 1 for p53 staining or 0 for no stain and plotted using Prism 4a (GraphPad Software) to obtain a statistical value.

\textbf{Establishment and cultivation of primary lung adenocarcinoma cell lines}

For animals >15 weeks after intranasal administration of AdenoCre treatment and having dissectable tumor nodules, the nodules were isolated using sterile razor blades, digested with collagenase/dispase (Sigma) at 4 mg/ml in RPMI for 1 h at 37°C and resuspended with 20% fetal calf serum in RPMI. The cells were seeded in freshly prepared fibronectin/vitrogen-coated plates and passaged by trypsinization.

\textbf{Chromosome analysis}

Metaphase spreads were made from exponentially growing early passage cell lines incubated with 0.1 μg/ml colcemid (Gibco, Carlsbad, CA) for 3–4 h, trypsinized, spun down in phosphate-buffered saline, incubated with hypotonic solution (0.075 M KCl) for 20 min at 37°C, fixed with methanol-glacial acetic acid (3:1 vol:vol) and dropped onto a clean wet slide from a height of 18–24 inches. For telomere visualization, metaphases were fixed with 4% formalin and hybridized with telomere-specific fluorescent dye-labeled (CCCTAA)n3 peptide nucleic acid probe (Applied Biosystems, Foster City, CA) (18). For spectral karyotyping, metaphase spreads were incubated with mouse SkyPaint kit probe (Applied Spectral Imaging, Vista, CA) and counterstained with 4′,6-diamidino-2-phenylindole and then analyzed as described previously (19). Briefly, raw images were assigned red, green and blue colors for specific spectral ranges and chromosomes were identified and visualized using SKY View software (Applied Spectral Imaging). Chromosome aberrations were identified in 10–15 metaphases from each cell line under the nomenclature rules from the Committee on Standard Genetic Nomenclature for Mice.

\textbf{Results}

We generated compound mutant mice to test the functional interactions between telomere dysfunction, the telomere checkpoint and activated \textit{K-rasG12D} in a mouse model of lung cancer. Our breeding scheme involved crosses between generation four (G4) \textit{mTerc}+/−, p53+/− mice with \textit{mTerc}−/− (G0) \textit{K-rasG12D} p53+/− animals in order to obtain two cohorts—denoted \textit{G0i} (\textit{mTerc}−/−) and \textit{G5i} (\textit{mTerc}+/−)—that differed in telomere function and p53 checkpoint status (supplementary Figure 1, available at \textit{Carcinogenesis} Online) (20). The \textit{G5i K-rasG12D} mice were confirmed to harbor constitutional signs of telomere dysfunction including reduced body weight, frailty, gonadal atrophy, increased intestinal cryp atrophy and increased end to end chromosomal fusions in bone marrow cells, whereas \textit{G0i K-rasG12D} littermates showed no such degenerative phenotypes or cytogenetic aberrations (supplementary Figure 2, available at \textit{Carcinogenesis} Online) (20).

Telomere dysfunction prolongs survival of mice with \textit{K-rasG12D}-driven lung cancer

To assess \textit{K-rasG12D}-initiated lung tumor initiation and progression as a function of telomere and p53 status, the latent \textit{K-rasG12D} allele was activated in mice aged 6–8 weeks via intranasal administration of AdenoCre at 1 × 10^7 plaque forming unit as described previously (17). This well-established protocol produces precursor lesions that progress into tumors >5 mm^2 by 14 weeks after AdenoCre administration, resulting in death at a median age of 20–24 weeks (21). The highly reproducible and well-defined kinetics and histopathological evolution of this model provides a framework to monitor the modulating effects of telomere dysfunction on tumor initiation, progression and survival. Median life span of \textit{G5i K-rasG12D} p53+/− mice was significantly longer than the \textit{G0i K-rasG12D} p53+/− mice (30.5 weeks versus 24 weeks, P = 0.0016) (Figure 1), an observation consistent with tumor-suppressive activity of telomere dysfunction with...
an intact p53 checkpoint (5). In cohorts heterozygous for p53, the 
overall survival differences between G0i K-ras<sup>G12D</sup> p53<sup>+/−</sup> and G5i K-ras<sup>G12D</sup> p53<sup>+/−</sup> mice were less pronounced than in the p53<sup>+/+</sup>
comparisons, suggesting that p53 heterozygosity may attenuate telo-
mere-induced checkpoint responses and/or reflect the increased like-
lihood of loss of the remaining wild-type allele of p53 in genomically
unstable cells (Figure 1).

Telomere dysfunction results in higher grade lung cancers
Next, we studied the impact of p53 checkpoint status and telomere
dysfunction on the tumor burden and histopathological progression of
these K-ras<sup>G12D</sup>-driven lung cancers. Detailed quantitative analyses of
the overall tumor load per lung as a function of time after AdenoCre
exposure, as well as overall tumor number as a function of size, did
not reveal any significant differences among the G0i and G5i
K-rasG12D p53+/+ or p53−/− cohorts (supplementary Figures 3 and 4, available at Carcinogenesis Online). In G0i and G5i K-rasG12D p53+/− mice, early time analyses showed comparable tumor grades—atypical adenomatous hyperplasia at 6 weeks and low-grade adenomas at 12 weeks—and these adenomas were significantly larger in G0i K-rasG12D p53+/+ mice (size range 1–5 mm²) compared with G5i K-rasG12D p53+/− mice (all <2 mm²) (Figure 2A). Notably, however, despite the decreased tumor burden in the G5i K-rasG12D p53+/+ mice at earlier time points, by 27 weeks after AdenoCre treatment, these tumors were similar in size to the G0i K-rasG12D p53+/− lung tumors but exhibited more aggressive malignant features including pleomorphic nuclei, hyperchromatism, multinucleate giant cells, atypical mitotic figures and more invasive growth (Figure 2B). These tumors in the G5i K-rasG12D p53+/− mice also showed pronounced stromal desmoplasia, a feature that was not present in tumors of the G0i K-rasG12D p53+/− cohort.

The importance of p53 in malignant progression and telomere checkpoint responses prompted analysis of p53 status at late time points (24–30 weeks after AdenoCre treatment) in the tumors arising from various cohorts. Comparison of the G0i and G5i K-rasG12D p53+/+ tumors >3 mm in diameter revealed comparable frequencies of loss of p53 expression in mice examined (5 of 15 tumors in G0i K-rasG12D p53+/+ versus 2 of 7 in G5i K-rasG12D p53+/−; Figure 2C, right panel). In contrast, prominent differences in p53 expression patterns were observed in comparisons of late-stage G0i and G5i K-rasG12D p53+/− tumors. Whereas 10 of 19 advanced tumors in G0i K-rasG12D p53+/− mice showed nuclear expression of p53, p53 expression was not detected in most advanced tumors in the G5i K-rasG12D p53+/− cohort (3 of 27 tumors; Figure 2C, P = 0.0015, see Materials and Methods). These results suggest that telomere dysfunction in G5i K-rasG12D p53+/− lung tumors creates selective pressure for p53 loss of heterozygosity (LOH), leading to accelerated tumor progression at late stages.

Telomere dysfunction causes apoptosis and a proliferative block in K-rasG12D-expressing lung tumors

Telomere deficiency can restrain tumor progression by inhibiting cell proliferation or provoking apoptosis (22–24). We selected tumors of comparable sizes from the various genotypes and assessed proliferation rates (by Ki-67 staining) and apoptosis (by TUNEL). A significant increase in tumor cell proliferation was observed at time points of 6–8 weeks and 27–30 weeks, following AdenoCre treatment in the G0i K-rasG12D p53+/+ group compared with the G5i K-rasG12D p53+/− group (Figure 3A, P = 0.018 and 0.037, respectively). Similar increases in tumor cell proliferation were observed in the G0i K-rasG12D p53+/− group relative to the G5i K-rasG12D p53+/− mice at the 6–8 weeks time point and comparable trends were evident at the later time points (Figure 3B; histology is shown in Figure 2D and E). With respect to apoptosis, telomere dysfunction was associated with increased intratumoral apoptosis across all time points, regardless of p53 status (see Figure 3C and D; histology is shown in Figure 2D and E). The observed impaired proliferation and increased apoptosis in the telomere-dysfunctional cohorts may contribute to observed smaller tumors and increased survival in G5i K-rasG12D p53+/+ and p53+/− mice.

Lang cancer cell lines with telomere dysfunction show genomic instability

Loss of telomere function in mTerc null mice has been shown to promote chromosomal structural rearrangements, end fusions and aneuploidy in normal and cancer cells (14,25,26). We used conventional cytogenetic and spectral karyotyping analyses to examine the cytogenetic profiles of early passage tumor cell lines derived from age-matched G0i and G5i K-rasG12D p53+/− mice. Interestingly, it took a longer time to establish G0i K-rasG12D p53+/− cell lines than the corresponding G5i cell lines. This may have been due to G5i cells undergoing p53 LOH at an earlier time than the G0i cells in culture.

![Fig. 3. Late generation G5i K-rasG12D p53+/− lung tumors exhibit similar proliferation but significant increase in apoptosis compared with G0i counterparts. Proliferation was measured by scoring five similar high-power tumor areas of Ki-67 immunohistochemically stained lung sections from K-rasG12D p53+/+ (A) and K-rasG12D p53+/− (B) mice at indicated time points. Apoptosis was measured similarly from TUNEL-stained lung tumors from K-rasG12D p53+/+ (C) and K-rasG12D p53+/− (D) mice.](https://academic.oup.com/carcin/article-abstract/29/4/747/2624519)
All the cell lines examined were verified by PCR to have sustained loss of the wild-type p53 allele (Figure 4A). PCR was also carried out on mouse tail DNA (and tumor from which the cell line was derived) DNA for p53 status comparison. All mice were initially heterozygous for p53 as shown by tail DNA PCR (Figure 4A). We also observed that the tumor DNA was heterozygous for p53 (Figure 4A). This may be due to stromal contamination of p53 heterozygous non-tumor cells and/or inclusion of p53 heterozygous cells within the tumor that has not yet undergone LOH as shown by p53 immunohistochemistry in Figure 2C. The G5i K-rasG12D p53+/− metaphase spreads showed increased frequency of P–P arm chromosomal fusions relative to G0i K-rasG12D p53+/− controls (Figure 4B; P < 0.0001). Further, rare Q–Q arm fusions were observed only in the G5i K-rasG12D p53+/− metaphases along with increased frequency of translocations, tricentric chromosomes and aneuploidy (Figure 4C). These data show that telomere dysfunction can promote high levels of genomic instability in this model, although clonal expansion of specific translocations was not observed (Figure 4C). Together, these data show that telomere dysfunction can drive genomic instability in lung cancer, although there is a notable lack of clonal events.

**Invasion and metastasis in the murine lung adenocarcinomas of G5i K-rasG12D p53+/− mice**

Invasion of local lymph nodes and metastatic spread are pathological hallmarks of human lung cancer progression. Adenocarcinomas arising in aged G5i K-rasG12D p53+/− mice showed invasion to the lymph nodes in 8 of 18 animals and to the heart muscle in 1 animal (Figure 5). In contrast, only 1 of 20 age-matched G0i K-rasG12D p53+/− mice showed metastatic lymph node invasion even though, as noted above, the tumors in this cohort were generally larger in size. Loss of p53 expression was observed in all metastases analyzed (Figure 5). Pan-cytokeratin staining confirmed that the metastatic cells were of epithelial origin (Figure 5). Hence, despite the decreased tumor burden and lower rates of cell proliferation, telomere-dysfunctional lung cancers show a markedly increased metastatic potential. Overall, genomic instability may play an important role in promoting metastasis.

**Discussion**

Genomic instability resulting from telomere dysfunction or inactivated DNA damage checkpoints is a defining property of human

**Fig. 4.** Cell lines generated from the lung tumors of G5i and G0i K-rasG12D p53+/− mice exhibit loss of p53 heterozygosity but increase in cytogenetic abnormality in G5i K-rasG12D p53+/− lung tumor cell lines. (A) Several lung tumor cell lines generated from G5i and G0i tumors were analyzed by PCR for the presence of p53 gene. All cell lines demonstrate loss of p53 even though it is heterozygous in the tail and primary tumor DNA (KO, knockout band; WT, wild-type band). (B) Metaphase spreads made from the tumor cell lines indicate that G5i K-rasG12D p53+/− tumors have increased fusion and aneuploidy. Increased cytogenetic abnormality in the G5i K-rasG12D p53+/− cells is exhibited by the significant increase in the added P–P and Q–Q arm fusions. (C) Spectral karyotyping-stained cells from G5i K-rasG12D p53+/− mice (left and middle panels) show aneuploidy and increased translocations and fusions (outlined) compared with G0i K-rasG12D p53+/− cells (right panel). Top row is light microscopic images of 4’,6-diamidino-2-phenylindole-stained metaphase spreads and bottom row consists of same spread stained by spectral karyotyping.
carcinomas. Although telomere erosion can be a potent block to cellular growth in vitro and in vivo, telomere dysfunction and ensuing chromosome bridge–fusione–breakage cycles can also result in tumor-promoting gene amplifications, deletions and translocations. This chromosomal instability is not effectively recapitulated in most mouse models of cancer. In this study, we investigated the contribution of telomere dysfunction to lung cancer pathogenesis. These studies revealed that telomere dysfunction is limiting for initial K-rasG12D-driven tumor expansion, yet when the telomere dysfunction-induced checkpoint is diminished by the loss of p53, malignant progression can occur, culminating in metastasis, indicating that telomere dysfunction in this context can facilitate tumor progression. Equally important, we have now generated a mouse model of lung cancer that more closely recapitulates the human disease.

By employing a modified breeding scheme (20), we generated cohorts of K-rasG12D-bearing mice differing in telomere function status. Lung tumors in a G0i K-ras-activated p53 heterozygous mouse resembled those seen previously in K-rasG12D single mutant mice (27), showing low degrees of nuclear atypia and a low propensity for metastasis. Consistent with previous reports (27–29), loss of p53 promotes histological progression of K-rasG12D-driven lung tumors. Importantly, our studies show that although telomere dysfunction prevents early neoplastic growth, leading to longer tumor-free survival, it nonetheless promotes tumor progression in p53 heterozygous K-rasG12D mice, including the formation of local metastases. p53 was inactivated in these tumors, suggesting that telomere dysfunction contributes to progression in K-rasG12D-driven tumors by inducing genomic instability resulting in loss of p53. As p53-null mice rarely manifest lung tumors within 6 months, these results imply that K-ras activation is essential for tumorigenesis but loss of p53 in combination with telomere dysfunction greatly promotes progression and metastasis of adenocarcinomas.

Several lines of evidence indicate that the p53 checkpoint is central to the cellular response to telomere dysfunction. Prior studies in telomerase-deficient p53-null mice indicate that without the DNA damage checkpoint function of p53, cells with telomere dysfunction may have enhanced oncogenic capacity (5). Moreover, mutation of p53 in mice with telomere attrition promotes emergence of epithelial cancers (14). In contrast, telomerase-deficient mice with mutations in the Ink4a/Arf, Apcmin or Atm tumor suppressors—but with intact p53—have reduced incidence of cancer (6,30–32). In our model system, the importance of telomere dysfunction in preventing tumor growth and progression was most evident by the reduced proliferation in the early lesions (6–8 weeks) and the increased apoptotic rate seen in nearly all time periods in p53 wild-type mice. Further, a reduction in apoptosis in G5i K-rasG12D p53–/– tumors correlated with loss of p53 at late time points. This is consistent with the hypothesis that cells with shortened telomeres experience crisis and undergo apoptosis when the p53 checkpoint is intact, but progress toward malignancy in the absence of p53 (5,6,30,33,34).

Recent reports suggest that p53-dependent cellular senescence pathways inhibit spontaneous tumorigenesis in mTerc-null mice (35,36). We did not observe many senescent cells via β-galactosidase staining in any of the lungs from the different cohorts (data not shown). However, it should be noted that in this model system, it was difficult to precisely assess contribution of senescence-induced tumor inhibition due to confounding β-galactosidase staining from numerous macrophage infiltration of most lung areas (37). Our data are also consistent with the lack of senescence associated with K-ras activation at physiological levels in mouse embryonic fibroblasts (38).

In summary, our study provides further evidence that telomere dysfunction can serve as both a potent tumor suppressor as well as promoter, depending on the status of the telomere dysfunction-induced checkpoint, in the lung epithelial compartment. The model outlined here provides an excellent platform to investigate the genetic alterations essential for driving the changing role in telomere length. Further work will be required to dissect the precise molecular mechanisms by which eroded telomeres can have such opposing functions in tumor development.

Supplementary material
Supplementary Figures 1–4 can be found at http://carcin.oxfordjournals.org/.

Funding
National Cancer Institute (CA122794); National Institute on Aging (AG027757); Department of Defense; Cecily and Robert Harris Foundation; Flight Attendant Medical Research Institute.

Acknowledgements
We thank Drs Robert F.Padera Jr, Lucian R.Chirieac and the Harvard Lung Specialized Programs Of Research Excellence group for advice and helpful discussions. We thank the laboratory of Dr Tyler Jacks for the use of Bioquant software and microscope. We thank Elena Ivanova, Christine Lam and Mei Zheng for technical support.

Conflict of Interest Statement: None declared.

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