

Roles of tumor suppressor and telomere maintenance genes in cancer and aging—an epidemiological study

Jian Gu, Margaret R. Spitz, Hua Zhao, Jie Lin,
H. Barton Grossman¹, Colin P. Dinney¹ and Xifeng Wu*

Department of Epidemiology and ¹Department of Urology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX, USA

*To whom correspondence should be addressed. Tel: +1 713 745 2485;
Fax: +1 713 792 4657
Email: xwu@mdanderson.org

Advanced age is strikingly linked to increased incidence of cancer. To gain insight into the mechanism underlying the association between increased cancer incidence and aging in normal human physiological conditions, we used a case-control design and measured the mRNA expression levels of *p53*, *ATM*, *hTERT* and *TRF2*, the four major protectors of genomic integrity, in isolated peripheral blood lymphocytes from 202 confirmed bladder cancer (BC) patients and 199 healthy controls. Significant age effects on expression levels were observed. When we divided the study subjects into three age groups (<57, 57–65 and ≥65), the expressions of *p53*, *ATM* and *TRF2* significantly decreased with advancing age in cases (*P* for trend ≤0.001, 0.01 and 0.01 for *p53*, *ATM* and *TRF2*, respectively). In controls, however, *p53* expression significantly increased with advancing age (*P* for trend = 0.05). Among subjects ≥65 years of age, the expressions of *p53*, *ATM* and *TRF2* were significantly lower in cases than in controls (*P* = 0.003, 0.04 and 0.05 for *p53*, *ATM* and *TRF2*, respectively), suggesting that attenuated genomic maintenance mechanisms lead to increased cancer risk in older individuals. When we dichotomized our study population at the median age of study subjects (61 years old), low *p53* expression was associated with a significantly increased BC risk in older people (OR = 2.27, 95% CI = 1.00–5.16). In addition, older subjects without detectable *hTERT* expression had a significantly reduced BC risk (OR = 0.41, 95% CI = 0.17–0.99). Our study provides the first epidemiologic evidence that the increased genomic instability resulting from the combination of telomere dysfunction, impaired *ATM*- and *p53*-mediated DNA damage, and/or telomere dysfunction response pathway contributes to increased cancer incidence in the elderly population.

Introduction

In the US, cancer is the second leading cause of death after heart disease. The majority of cancers affect older people disproportionately. According to the NCI Surveillance, Epidemiology and End Results (SEER) program data for 1995–1999, close to 60% of all newly diagnosed malignant tumors and 71% of all cancer deaths occur in persons

≥65 years of age. The age-adjusted cancer incidence rate for this age group is 10 times greater than the rate for persons under 65 years. It is apparent, therefore, that aging is a major risk factor for cancer. Although the reason for the connection between increased cancer risk and aging is not well understood, it is becoming increasingly clear that cancer and aging are connected in several ways at the cellular and molecular levels. Genomic instability is considered a major causal factor for both cancer and aging. Age-related accumulation of somatic DNA mutations—which may activate and/or inactivate specific genes involved in key cellular functions such as DNA repair, apoptosis and cell cycle control—is likely a major contributing factor for the increased cancer incidence with age. Evidence from animal studies and human genetics research suggests that tumor suppressor genes may also play distinct roles in the mammalian aging process. Tumor suppressor genes can be generally grouped into two classes: caretakers, which act to protect the genome from damage and mutation, and gatekeepers, which function to prevent the proliferation of potential cancer cells by inducing either cellular senescence or apoptosis (1). Proteins encoded by caretaker genes include all major DNA repair pathway components, such as ATM (DSB repair), the XP family (NER), the RecQ family of DNA helicases (WRN, BLM, etc.) and telomere maintenance proteins. Caretaker genes mostly function to protect the organism from cancer and slow the aging process. Therefore, increased cancer incidence and premature aging usually accompany many human genetic diseases that result from defects in caretaker genes, such as ataxia–telangiectasia (A–T, ATM mutation), xeroderma pigmentosum (XP gene mutation) and Werner's syndrome (WRN mutation). In contrast, gatekeeper genes, the most prominent of which is *p53*, may contribute directly to the aging process. It is well known that *p53* is the most mutated gene in human cancers, *p53* deficiency favors cancer development and forced overexpression of wild-type *p53* elicits strong anti-cancer effects by inducing cell cycle arrest and/or apoptosis. Owing to two surprising findings, the study of *p53*'s role in the aging process is receiving increasing attention. Tyner *et al.* (2) and Maier *et al.* (3) independently created two different lines of transgenic mice with hyperactive *p53* alleles. Despite the strong cancer resistance of these mice, both lines displayed shortened life spans and accelerated aging phenotypes, thus providing support for the antagonistic pleiotropy theory of aging (4). Antagonistic pleiotropy has been used to explain how genetic traits can have both beneficial and deleterious effects on a species (4). By virtue of its conference of protection against cancer at the cost of accelerated aging, *p53* may qualify as an antagonistic pleiotropic trait.

Many studies, from *in vivo* animal models and *in vitro* cell culture, have shown that aberrant gene expressions play significant roles in both cancer and aging. However, data from these studies may not be fully extrapolative to normal human aging *in vivo*. Welle *et al.* (5) found that only about one-third of genes showed similar age-related change in expression in mice and

Abbreviations: BC, bladder cancer; FP, forward primer; RP, reverse primer.

humans. For *in vitro* cultured human cells, growth conditions may affect gene expression profoundly. In this bladder cancer (BC) case-control study, we used real-time PCR to measure the expression of four critical genomic maintenance genes in non-cultured peripheral blood lymphocytes directly isolated from 202 confirmed BC patients and 199 healthy controls. We selected *p53*, *ATM*, *hTERT* and *TRF2* for analysis because evidence suggests that the *ATM-p53*-telomere axis plays a key role in both aging and cancer (6,7). *ATM* is a caretaker gene mutated in the autosomal recessive disorder ataxia-telangiectasia, which is characterized by progressive neurological degeneration, growth retardation, accelerated telomere shortening, genomic instability, premature aging and increased risk of cancer (8). *hTERT* is the rate-limiting catalytic subunit of telomerase. *TRF2* is a major telomere maintenance protein that protects chromosome ends by maintaining the correct structure and repressing chromosome end-to-end fusion. We hypothesize that there might be deficient expressions of critical genomic maintenance genes in elderly people, which predispose them to BC. Our aim was to evaluate gene expression variations in cancer and aging under normal physiological conditions and gain insight into the potential mechanism of increased cancer incidence in elderly people. The relatively large sample size allows us to perform stratified analysis and determine the expression pattern of these critical genes in different age groups among cancer patients and controls.

Materials and methods

Study population

For the study, 202 newly diagnosed, histologically confirmed BC patients were recruited from The University of Texas MD Anderson Cancer Center and the Methodist Hospital (Houston, TX) between 1999 and 2002. These patients had been diagnosed within 1 year of recruitment and had not received chemotherapy, radiotherapy and immune therapy before enrollment. There were no age, gender, ethnicity or tumor stage restrictions. 199 control subjects with no prior history of cancer (except non-melanoma skin cancer) were recruited from Kelsey Seybold, the largest multi-specialty managed-care physician group in the Houston metropolitan area. Control subjects were matched to the case patients by age (± 5 years), sex and ethnicity. Informed consent was obtained from all study participants before the collection of epidemiological data by trained MD Anderson staff interviewers. Data were collected on demographics, smoking history, alcohol consumption, family history of cancer, medical history, and occupational history and exposures.

Sample collection

Blood samples were collected before any treatment. Immediately after the interview, a 40 ml sample of blood from each participant was collected into heparinized tubes. The tubes were first coded with a unique identification number to ensure that laboratory personnel were blinded to case-control status and then transported immediately to the laboratory where the specimens were processed. Lymphocytes were isolated by Ficoll-Hypaque centrifugation and aliquots of 4×10^6 isolated lymphocytes per vial were stored in liquid nitrogen as described previously (9).

RNA isolation

Total RNA was isolated from lymphocytes using the micro-column-based E.Z.N.A. RNA kit (Omega Bio-Tek, Doraville, GA) according to the manufacturer's protocol. The kit utilizes the reversible binding property of RNA to HiBind matrix, a silica-based material. Briefly, 4×10^6 isolated lymphocytes were lysed in 400 μ l lysis buffer and mixed with 400 μ l 70% ethanol; then, the mixtures were applied onto HiBind RNA spin columns. After two rounds of column-washing with washing buffers, total RNA was eluted with 50 μ l DEPC-treated water.

Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was performed with a two-step procedure. In the RT step, 200 ng of total RNA per sample was reverse transcribed into cDNA using the MultiScribe reverse transcriptase (Applied Biosystems, Foster City, CA). Each reaction (20 μ l) contained total RNA, $10 \times$ RT buffer (2 μ l);

200 μ M each of dATP, dCTP, dGTP and dUTP; 5 mM $MgCl_2$; 2.5 U of MultiScribe reverse transcriptase; 1 U of RNase inhibitor, and 2.5 μ M of the random hexamer. The reaction occurred at 42°C for 30 min, 99°C for 5 min and 4°C for 5 min. An aliquot of 2 μ l of RT product from each sample was used for each gene in the subsequent quantitative real-time PCR amplification. The probes and primers used for real-time RT-PCR were designed using the Primer Express software (version 2.0; Applied Biosystems). To avoid amplification of the contaminating residual genomic DNA, the probe and primer sets for each gene were designed around the junction region of two exons so that they were mRNA-specific. The sequences of primers and probes are as follows: *p53*, forward primer (FP): CCTATGGAACTACTTCTGAAAACAA, reverse primer (RP): ACAGCATCAAATCATCCATTGC, Probe: TCTGTC-CCCCTTGCCGTCCTCA; *ATM*, FP: TTATTTACTGGGTCAGCCTGCA, RP: GAACATACTGGTGGTTCAGTGCCA, Probe: ACCTTCATGTCCTGCA-GTATGCTGTTTGACT; *hTERT*, FP: TGCAGAGCGACTACTCCAGCTA, RP: GAAGCCGCGGTTGAAGGT, Probe: CCCGGACCTCCATCAGAGC-CAGTC; *TRF2*, FP: ACCAGGGCCTGTGGA AAAAG, RP: GGTGGTTG-GAGGATCCCGTA, Probe: CACCCAGAGA ACCCGCAAGGCAG.

Human total RNA was used as a relative standard and the human *GAPDH* gene was used as an internal control to normalize input RNA amount, RNA quality and reverse transcription efficiency. The sequences of primers and probe for *GAPDH* are as follows: FP: AAGGCTGAGAACGGGAAGC, RP: GAGGGATCTCGCTCCTGGA, Probe: TGTCATCAATGGAAATCCCAT-CACCATC. Real-time PCR was performed using the ABI Prism 7700 Sequence Detection System according to the manufacturer's protocol. Typical amplification mixtures (25 μ l) contained the sample DNA (or cDNA); $10 \times$ TaqMan buffer (2.5 μ l); 200 μ M of each dATP, dCTP, dGTP and 400 μ M dUTP; 5 mM $MgCl_2$; 0.65 U of AmpliTaq Gold; 0.25 U of AmpErase uracil *N*-glycosylase; 200 nM each primer and 100 nM probe. The thermal cycling conditions consisted of 1 cycle for 2 min at 50°C and for 10 min at 95°C, and 50 cycles for 15 s at 95°C and for 1 min at 60°C. The relative quantification values were obtained automatically based on the standard curve of human control RNA for each gene. The quantitative PCRs were performed in duplicate for each sample, and the mean was used as the relative quantification value. The tested gene expression level was then normalized to *GAPDH* gene expression.

Statistical analysis

Among cases and controls, smoking status was defined as follows: 100 cigarettes or more in his/her lifetime = ever-smoker; smoking cessation of at least 1 year prior to diagnosis for cases and 1 year prior to interview for controls = former. Pack-years were computed as the number of cigarettes/day divided by 20 and then multiplied by the number of years smoked. The χ^2 -test was used to assess differences between cases and controls in the distributions of gender, ethnicity and smoking status, and the Student's *t*-test was used to assess differences between cases and controls for age, pack years and gene expression. Odds ratios (ORs) with 95% confidence limits were calculated as estimates of the relative risk by dichotomizing each gene expression at the 75th percentile of controls. The crude ORs were calculated by the Woolf method. The adjusted ORs were calculated by logistic regression to control for age, ethnicity and smoking status, wherever appropriate. Pearson's correlation coefficient was used to measure the correlation between the gene expression and age. The STATA statistical software (College Station, TX) was used to perform all statistical analyses for this study.

Results

Characteristics of the study population

A total of 202 confirmed BC cases and 199 controls were included in the current study. Owing to the small numbers of subjects from minority groups, only Caucasians were included in the data analysis (190 cases and 192 controls). Table I shows selected characteristics of the study population. The cases and controls were well matched on gender and age. As would be predicted, the cases had significantly higher percentage of current smokers (32%) than controls (9%), half of whom were never smokers.

mRNA expression levels in cases and controls and correlation with age

As shown in Table II, overall, there were no significant differences in levels of gene expressions by case-control status. However, an evident age effect on gene expressions was

observed. When we divided the study subjects into three age groups (<57, 57–65 and ≥65), the expressions of *p53*, *ATM* and *TRF2* significantly decreased with advancing age in cases (P for trend ≤0.001, 0.01 and 0.01 for *p53*, *ATM* and *TRF2*, respectively). In controls, however, *p53* expression significantly increased with advancing age (P for trend = 0.05). Among subjects ≥65 years of age, the expressions of *p53*, *ATM* and *TRF2* were significantly lower in cases than in controls (P = 0.003, 0.04 and 0.05 for *p53*, *ATM* and *TRF2*, respectively). *hTERT* expression was very low and showed no obvious difference either between age groups (P for trend = 0.57) or between cases and controls in any age group. When the correlation between mRNA expression and age as a continuous variable was assessed, significant inverse correlations were observed between *p53* and age (P = 0.002), *ATM* and age (P = 0.006) and *TRF2* and age (P = 0.003) in the cases (Figure 1). The controls showed a trend of increasing *p53* expression with advancing age, although the correlation did not reach statistical significance (P = 0.18), probably owing to the high heterogeneity of expression among individuals

Table I. Distribution of select characteristics by case-control status

Variables	Cases ($n = 190$)	Controls ($n = 192$)	P -value
Gender (%)			
Men	159 (84%)	160 (83%)	
Women	31 (16%)	32 (17%)	0.93
Smoking status (%)			
Never	46 (24%)	101 (53%)	
Former	83 (44%)	73 (38%)	
Current	61 (32%)	18 (9%)	<0.001
Age, median (range)	61 (31, 86)	61 (33, 85)	0.86
Pack-years, median (range)	38.5 (0, 176)	22 (0.1, 104)	<0.001

Table II. mRNA expression levels between cases and controls

Variables	Cases ($n = 192$)		Controls ($n = 190$)		P -value
	Median (range)	P -value	Median (range)	P -value	
Overall					
<i>p53</i>	2.01 (0.28, 4.83)		2.09 (0.17, 11.95)		0.81
<i>ATM</i>	6.25 (1.18, 23.73)		6.57 (1.45, 24.1)		0.59
<i>hTERT</i>	0.15 (0, 0.87)		0.14 (0, 1.04)		0.78
<i>TRF2</i>	0.84 (0.16, 2.20)		0.88 (0, 2.64)		0.76
Stratified by age					
<i>p53</i>					
<57	2.16 (0.45, 4.55)	Ref.	1.95 (0.22, 4.54)	Ref.	0.04
57–65	2.11 (0.45, 4.43)	0.4	2.08 (0.31, 4.60)	0.4	0.76
≥65	1.65 (0.28, 4.83)	0.003	2.23 (0.17, 11.95)	0.049	0.003
		P for trend < 0.001		P for trend = 0.05	
<i>ATM</i>					
<57	6.6 (1.18, 23.73)	Ref.	6.40 (1.45, 18.3)	Ref.	0.87
57–65	6.5 (1.24, 16.11)	0.76	6.71 (1.56, 20.8)	0.74	0.7
≥65	5.22 (1.27, 19.3)	0.01	6.66 (1.50, 24.1)	0.77	0.04
		P for trend = 0.01		P for trend = 0.85	
<i>hTERT</i>					
<57	0.16 (0, 0.79)	Ref.	0.16 (0, 0.59)	Ref.	0.84
57–65	0.14 (0, 0.87)	0.57	0.13 (0, 1.04)	0.78	0.54
≥65	0.15 (0, 0.54)	0.53	0.12 (0, 0.51)	0.15	0.43
		P for trend = 0.57		P for trend = 0.14	
<i>TRF-2</i>					
<57	0.97 (0.45, 1.88)	Ref.	0.91 (0.45, 1.89)	Ref.	0.34
57–65	0.83 (0.16, 1.77)	0.036	0.84 (0.17, 1.59)	0.13	0.77
≥65	0.77 (0.23, 2.2)	0.008	0.93 (0, 2.64)	0.8	0.05
		P for trend = 0.01		P for trend = 0.81	

(Figure 1). We also performed multiple linear regression to further elucidate the relationship between gene expression and age while adjusting for confounding of smoking and gender. Consistent with the results obtained from the correlation analysis, multiple linear regression showed that the expression of *p53*, *ATM* and *TRF2* significantly decreased with increasing age in cases (P = 0.003, 0.006 and 0.012, respectively). In controls, there was a trend of borderline significance for increasing gene expression with increasing age for *p53* (P = 0.08).

mRNA expression levels in controls by smoking status and in cases by tumor stage

To evaluate the impact of important confounders on mRNA expression, we performed stratified analyses in controls by smoking status and in cases by tumor stage (Table III). There were no significant differences on mRNA levels of any of these four genes among never, former and current smokers. The cases were grouped into two categories, superficial (stages T0 and T1) and invasive (stages T2–T4). There were no significant differences between these two groups in any mRNA level. We also compared the expression level in T0 and T1 stage superficial bladder cases and did not find any significant associations (data not shown). We did not have a detailed stage information in invasive cases and did not perform further analyses among this group.

Risk estimates based on mRNA expression

Table IV shows the association of BC risk with relative expression of the tested genes. For *p53*, *ATM* and *TRF2*, ORs with 95% confidence limits were calculated as estimates of the relative risk by dichotomizing each gene expression at the 75th percentile value in the controls. For *hTERT*, we estimated

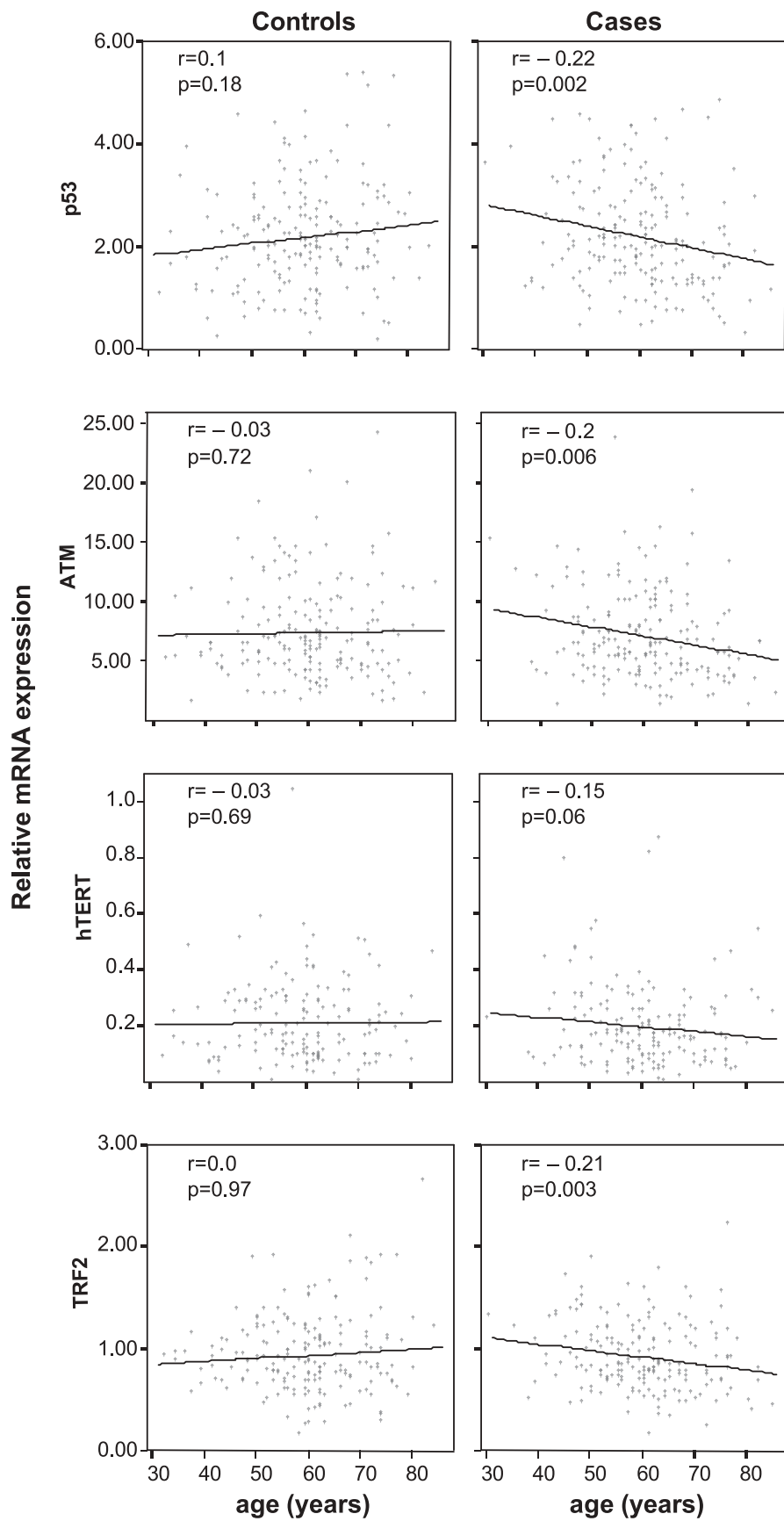


Fig. 1. Relation between gene expression and age in cases and controls. Pearson's correlation coefficient was used to assess correlation. See online supplementary material for a color version of this figure.

Table III. mRNA expression levels by smoking status in controls and tumor stage in cases

Genes	Controls by smoking status, median (range)			<i>P</i> -value	Cases by stage, median (range)		
	Never	Former	Current		Superficial	Muscle-invasive	<i>P</i> -value
<i>p53</i>	2.02 (0.22–5.09)	2.36 (0.17–11.95)	1.78 (0.57–4.60)	0.22	2.13 (0.31–4.83)	1.93 (0.28–4.62)	0.38
<i>ATM</i>	6.51 (1.64–24.10)	6.71 (1.50–16.90)	5.81 (1.45–14.50)	0.61	6.32 (1.18–23.82)	5.92 (1.26–19.3)	0.3
<i>hTERT</i>	0.16 (0.0–1.04)	0.10 (0.0–0.56)	0.15 (0.0–0.40)	0.3	0.17 (0.00–0.65)	0.14 (0.00–0.86)	0.85
<i>TRF2</i>	0.88 (0.00–2.64)	0.88 (0.25–2.08)	0.82 (0.17–1.52)	0.89	0.86 (0.23–2.30)	0.90 (0.16–2.62)	0.64

Table IV. Risk estimate relative to mRNA expression

Variables	Adjusted ORs (95% CI)
Overall	
<i>p53</i>	1.36 (0.79, 2.34)
<i>ATM</i>	1.22 (0.69, 2.13)
<i>hTERT</i>	0.66 (0.36, 1.21)
<i>TRF2</i>	0.75 (0.42, 1.36)
Stratified by age	
<61 years old	
<i>p53</i>	0.80 (0.37, 1.77)
<i>ATM</i>	1.27 (0.57, 2.82)
<i>hTERT</i>	1.14 (0.46, 2.81)
<i>TRF2</i>	0.70 (0.30, 1.64)
≥61 years old	
<i>p53</i>	2.27 (1.00, 5.16)
<i>ATM</i>	1.14 (0.49, 2.63)
<i>hTERT</i>	0.41 (0.17, 0.99)
<i>TRF2</i>	0.72 (0.30, 1.70)

Adjusted for age, gender and smoking status, wherever appropriate.

the risk based on the number of subjects without detectable mRNA expression in cases and controls because relative *hTERT* expression was very low in normal lymphocytes (Table II) and a substantial percentage of subjects lacked detectable *hTERT* mRNA (33 out of 192 in controls and 24 out of 190 in cases). Overall, although not statistically significant, low expression of *p53* or *ATM* was associated with slightly increased BC risk (OR = 1.36 and 1.22, respectively), whereas no *hTERT* expression or low *TRF2* expression conferred a slightly reduced BC risk (OR = 0.66 and 0.75, respectively). When we dichotomized our study population at the median age of the study subjects (61 years old), low *p53* expression was associated with a significantly increased BC risk in older people (OR = 2.27, 95% CI = 1.00–5.16). The percentage of subjects without detectable *hTERT* expression in older subjects was 21.4% (22 out of 103) for controls and 10.2% (10 out of 98) for cases, indicating that older subjects without detectable *hTERT* expression had a significantly reduced BC risk (OR = 0.41, 95% CI = 0.17–0.99).

Correlations of mRNA expression among the four genes in cases and controls

Since all four of these genes play major roles in maintaining genomic integrity and there is an intricate functional interplay among them as reported in the literature, we assessed whether their expression levels were correlated. As shown in Table V, there were significant correlations in mRNA expressions in both controls and cases. For example, in controls, the correlation coefficients between *p53* and *ATM*, *hTERT* and *TRF2* were 0.40 ($P < 0.001$), 0.22 ($P = 0.006$) and 0.52 ($P < 0.001$), respectively; in cases, the correlation coefficients were 0.48

Table V. Correlations of mRNA expression among the four genes in cases and controls

	<i>p53</i>	<i>ATM</i>	<i>hTERT</i>	<i>TRF2</i>
Controls				
<i>p53</i>	NA	0.4	0.22	0.52
<i>ATM</i>		NA	0.34	0.44
<i>hTERT</i>			NA	0.33
<i>TRF2</i>				NA
Cases				
<i>p53</i>	NA	0.48	0.21	0.62
<i>ATM</i>		NA	0.26	0.58
<i>hTERT</i>			NA	0.32
<i>TRF2</i>				NA

($P < 0.001$), 0.21 ($P = 0.005$) and 0.62 ($P < 0.001$), respectively. Similarly, significant correlations among *ATM*, *hTERT* and *TRF2* genes were observed in cases and controls. These data suggest that there are highly coordinated expressions in genomic maintenance genes in both healthy and disease states.

Discussion

This study yielded the following major findings: (i) *p53* expression increases in controls but decreases in cases with advancing age, and low *p53* expression confers a 2.3-fold increased BC risk in older subjects; (ii) there were concomitant reductions of *p53*, *ATM* and *TRF2* expressions with advancing age in cancer patients; (iii) the expressions of *p53*, *ATM*, *hTERT* and *TRF2* were significantly correlated in both normal and cancer states; (iv) a lack of detectable *hTERT* expression in older age is associated with a 59% reduced risk of BC cancer.

Cancer is a disease of aging and is increasing in magnitude as people live longer. Compelling evidence has shown that imperfect genome maintenance of DNA damage is partly responsible for aging (10,11). Experimental and epidemiological data consistently demonstrate that DNA repair capacity declines with increased age (12–14). On the other hand, numerous phenotypic assays have also shown that deficient DNA repair capacity and genomic instability confer higher cancer risk (9,15). Therefore, a deficient genome maintenance mechanism might be an important contributor to increased cancer incidences in an aged population. Defects in *p53*, *ATM* and *TRF2*—genes that maintain genomic integrity—ultimately cause cancer. *p53* is the most prominent genomic guardian

gene and plays a pivotal role in many critical cellular events related to human aging and cancer, including DNA damage, apoptosis, cell cycle control, telomere shortening and oxidative stress (16). *ATM* is a central player in DNA repair and checkpoint activation, which upon activation by double strand breaks, phosphorylates a plethora of downstream target proteins and promotes cell cycle arrest and DNA repair. TRF2 is a sequence-specific DNA binding protein that protects telomere termini and represses chromosome end-to-end fusion in cultured primary cells. The correlation of advancing age in cancer patients with the reduced expression of these three genes—*p53*, *ATM* and *TRF2*—acting in distinct genome maintenance pathways strongly suggests that compromised DNA repair and/or genotoxic attack response contribute to increased cancer incidence in elderly people.

A few recent studies showed that mRNA expression level of DNA repair or methylation-related gene in peripheral blood lymphocytes is associated with altered cancer risk. For example, reduced expression of mismatch repair genes and nucleotide excision repair genes confers significantly increased risk for head and neck cancer (17,18). Increased expression of MBD2, a gene involved in transcriptional repression and methylation, was associated with a significantly reduced BC risk (19). In this study, we found a significant lower expression of genomic maintenance genes in patients than in controls among individuals >65 years of age, suggesting that reduced mRNA expression in genomic maintenance genes is a predisposing factor for BC in elderly people. Biologically, decreased gene expression involved in DNA repair pathway is an important mechanism for age-related reduction in DNA repair capacity (13,14). It should be pointed out that mRNA level in peripheral blood lymphocytes is a cross-sectional measurement and may be affected by many factors, such as host characteristics, smoking status, disease stage and therapy. In this regard, a couple of studies showed that the mRNA levels of DNA repair genes were relatively consistent within an individual over a period of 1 year in peripheral blood cells from healthy controls (20,21). The inter-person variation is far larger than the intra-person variation, supporting the use of mRNA expression as potential biomarkers (20,21). We attempted to address these limitations by evaluating the impact of smoking status and tumor stage on the expression of these genes and did not find significant associations (Table III). Suzuki *et al.* (22) reported that Fas, Bcl-2 and p53 expressions in PBLs were not significantly different between normal individuals with chronic cigarette smoking and those without smoking. Nevertheless, mRNA expression is an intermediate event, which might be affected by genetic or environmental factors as well as disease process; therefore, a cause-effect relationship between reduced mRNA expression and increased cancer risk cannot be drawn from the descriptive data reported in this cross-sectional study.

Telomeres and telomerase clearly have implications for both cancer and aging (23–25). Telomere maintenance is essential for the protection of chromosome ends and genome integrity. Telomere dysfunction, including shortening in length or structural changes, has been shown to contribute to cancer and aging. Increased *hTERT* expression leading to telomerase activation is one of the most common features of human cancer: over 80% of all human cancers showed increased telomerase activities (26,27). The decline of telomere length with age in peripheral blood cells has also been reported (28). Recently Wu *et al.* have shown that telomere length in

peripheral blood cells from cancer patients is significantly shorter than those in healthy controls (9). In this study, we found that the expression of *hTERT* is consistently low in cases and controls, and a substantial percentage of subjects have no detectable *hTERT* expression. However, we found that compared with elderly patients, significantly more elderly controls had no detectable *hTERT* expression (10.2% versus 21.4% for patients and controls, respectively), which is consistent with many previous studies showing increased *hTERT* detection in the peripheral blood from cancer patients. The increase in *hTERT* detection in elderly cancer patients is likely a response of telomere shortening. The decline of *TRF2* expression coupled with advancing age in cases, telomere dysfunction resulting from both telomere shortening and telomere structure disruption may play significant roles in predisposing elderly people to cancer.

hTERT and *TRF2* are arguably the two most critical components in telomere maintenance. *p53* and *ATM* are two of the most critical players in telomere dysfunction-mediated cellular response pathways. Telomere shortening activates *p53* and results in growth arrest and/or apoptosis. Deletion of *p53* significantly attenuates the adverse effects of telomere attrition and, in conjunction with telomere dysfunction, accelerates carcinogenesis (6). Herbig *et al.* (29) showed that telomere shortening triggers senescence of human cells through the ATM-p53-p21 pathway. *ATM* deficiency and telomere loss act together to impair cellular and whole-organism viability and accelerates aging in double knockout mice (7). Telomere structure disruption by a dominant-negative *TRF2* allele and the ensuing de-protection of telomeres result in apoptosis via activation of the ATM and p53 DNA damage response pathway (30). These highly intertwined functional interactions among *p53*, *ATM*, *TRF2* and *hTERT* may explain their correlated expressions observed in this study. This association in both cases and controls suggests that genomic maintenance genes act in concert to protect cells against genotoxic attacks and that genome-wide deficiency in DNA damage response and genetic integrity may account for the increased cancer risk in the aged population.

In conclusion, this is the largest epidemiologic study to examine the roles of genomic maintenance genes in cancer by measuring expression levels of the genes. Our study suggests that the increased genomic instability resulting from the combination of telomere dysfunction and impaired ATM- and p53-mediated DNA damage and/or telomere dysfunction response pathway contributes to the increased cancer incidence in the elderly.

Supplementary material

Supplementary material is available online at: <http://carcin.oxfordjournals.org/>

Acknowledgement

This study was supported by the NCI grants CA 74880 and CA 91846.

Conflict of Interest Statement: None declared.

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Received December 20, 2004; revised May 3, 2005;
accepted May 10, 2005