Telomere Length and Recurrence Risk after Curative Resection in Patients with Early-Stage Non–Small-Cell Lung Cancer

A Prospective Cohort Study

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Background: We hypothesized that telomere length in peripheral blood would have significant predictive value for risk of recurrence after curative resection in non–small-cell lung cancer (NSCLC).

Methods: This prospective study included 473 patients with histologically confirmed early stage NSCLC who underwent curative therapy at MD Anderson Cancer Center between 1995 and 2008. Relative telomere length (RTL) of peripheral leukocytes was measured by real-time polymerase chain reaction. The risk of recurrence was estimated as hazard ratios (HRs) and 95% confidence intervals (CIs) using a multivariable Cox proportional hazard regression model.

Results: Median duration of follow-up was 61 months, and 151 patients (32%) had developed recurrence at time of analysis. Patients who developed recurrence had significantly longer mean RTL compared with those without recurrence (1.13 versus 1.07, $p = 0.046$). A subgroup analysis indicates that women had longer RTL compared with men (1.12 versus 1.06, $p = 0.025$), and the patients with adenocarcinoma demonstrated longer RTL compared with those with other histologic types (1.11 versus 1.05, $p = 0.042$). To determine whether longer RTL in women and adenocarcinoma subgroup would predict risk of recurrence, multivariate Cox analysis adjusting for age, sex, stage, pack year and treatment regimens was performed. Longer telomeres were significantly associated with higher risk of developing recurrence in women (hazard ratio [HR], 2.25; 95% confidence interval [CI], 1.02–4.96, $p = 0.044$) and adenocarcinoma subgroups (HR, 2.19; 95% CI, 1.05–4.55, $p = 0.036$). The increased risk of recurrence due to long RTL was more apparent in women with adenocarcinoma (HR, 2.67; 95% CI, 1.19–6.03, $p = 0.018$).

Conclusions: This is the first prospective study to suggest that long RTL is associated with recurrence in early stage NSCLC after curative resection. Women and adenocarcinoma seem to be special subgroups in which telomere biology may play an important role.

Key Words: Telomere, Telomere length, Telomerase, Early stage lung cancer, Non–small-cell lung cancer.

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Lung cancer is the leading cause of cancer-related death in the United States, with non–small-cell lung cancer (NSCLC) accounting for 87% of all cases.[1] Surgical resection in early stage offers the best chance of cure in NSCLC. However, even with complete resection, patients are at significant risk of recurrence and death from NSCLC.[2] Adjuvant cisplatin-based chemotherapy for completely resected early stage NSCLC can offer survival benefit,[3–5] but it is not clear which subgroup of patients would be at increased risk of developing recurrence. Hence, it is important to identify a high-risk group of patients who would most likely develop recurrence and potentially benefit from adjuvant chemotherapy. Likewise, a low-risk subgroup may be spared of toxic chemotherapy regimen. A biomarker-driven risk prediction model is necessary in molecularly heterogeneous NSCLC.

Telomere pathway is implicated in pathogenesis of various types of human cancer. Telomeres are specialized structures located at chromosome ends consisting of nucleotide repeats (TTAGGG)n and ordered protein complex that help maintain genomic structural integrity by protecting chromosome ends from degradation.[6] Progressive shortening of telomeres results in critically short telomeres leading to cellular crisis.[7] However, in cancer cells, up-regulation of telomerase is found in approximately 90% of human cancers leading to continued cellular proliferation.[8,9] Several studies have shown an association between shorter telomeres in peripheral leukocytes and risk of various types of cancer including lung cancer.[10,11]

There are a number of telomere-associated proteins that have essential roles in maintaining telomere length. For example, TRF1 and TRF2 are thought to be suppressors of telomere

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elongation.[12,13] POT1 binds the 3′ telomeric overhang and may regulate telomerase access.[14,15] TPP1 is identified as a regulator of POT1.[16] Human RAP1 is recruited to the telomere by TRF2 without directly binding to DNA[17] and targeting human RAP1 with small interference RNA leads to longer telomeres.[18] Such complexity suggests a highly regulated mechanism of telomere pathway that may affect clinical outcome.

There exists a significant interindividual variation in telomere length.[19,20] Furthermore, genome-wide association studies identified single nucleotide polymorphisms in genes encoding telomere-associated proteins that are implicated in maintaining leukocyte telomere length,[21,22] and risk of pancreatic,[23] skin,[24] and lung cancer.[25] We hypothesize that telomere length in peripheral leukocytes would have significant predictive value for recurrence following curative surgical resection in NSCLC, thereby guiding decision for adjuvant chemotherapy. To the best of our knowledge, this is the first investigation of the association between telomere length and clinical outcome in early stage NSCLC.

MATERIALS AND METHODS

Study Population

This prospective study included 467 patients with histologically confirmed NSCLC, who were enrolled from 1995 to 2008 in an ongoing epidemiologic lung cancer study at MD Anderson Cancer Center. Written informed consent was signed by all study participants, and the study was reviewed and approved by the Institutional Review Board of MD Anderson Cancer Center.

All the study participants had early stage disease (stage I and II) and underwent surgery or surgery plus chemotherapy (mostly platinum based) or radiotherapy. Each patient was interviewed by trained M. D. Anderson staff interviewers to collect comprehensive epidemiologic data using structured questionnaire. Follow-up data were abstracted from medical records for each patient. After the in-person interview, 40 ml blood sample was prospectively collected from each participant and sent to the laboratory for molecular analysis.

Telomere Length Assessment

High-quality genomic DNA was extracted from peripheral blood using the QIAamp Maxi DNA kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The details of telomere length assessment have been described previously.[26] The relative overall telomere length was assessed using a modified version of the real-time quantitative polymerase chain reaction (PCR) and was determined by the normalized ratio between the telomere repeat copy number and the single gene (human globulin) copy number to standardize between different runs. The PCR mixture (15 μl) for the telomere amplification consisted of 5 ng of genomic DNA, 1× SYBR Green Mastermix (Applied Biosystems, Grand Island, NY), 200 nmol/liter Tel-1, and 200 nmol/liter Tel-2. The PCR mixture for human globulin amplification consisted of 5 ng of genomic DNA, 1× SYBR Green Mastermix, 200 nmol/liter Hgb-1, and 200 nmol/liter Hgb-2. The thermal cycling conditions were 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 56°C (for telomere amplification) or 58°C (for Hgb amplification) for 1 minute. The PCRs for telomere and Hgb were done on separate 384-well plate, with the same samples in the same well positions. Negative controls, positive controls, a calibrator DNA, and a standard curve were included in each run. A six-point standard curve was created by diluting the reference DNA sample (the same DNA sample for all runs) using a twofold increment per dilution (from 20 to 0.625 ng) in each reaction.

Statistical Analysis

The primary end point was recurrence (time from diagnosis to recurrence or last follow-up). Recurrence was defined as evidence of local recurrence or new sites of involvement in lymph nodes or distant organ after curative resection. Patients who were lost to follow-up or who were alive at the end of the study without evidence of recurrence were censored. The association between telomere length and age was assessed by linear regression. Student's *t* test was used to assess the association of telomere length with recurrence. The multivariate Cox proportional hazard model while adjusting for age, sex, ethnicity, stage, pack year, and treatment regimens was used to assess the effect of telomere length on recurrence. Patients with previously diagnosed stage I and II disease who came to MD Anderson for treatment after developing recurrence were excluded from the Cox proportional hazard analysis of recurrence. All the statistical analyses above were performed using STATA software (version 10.1; Stata Corporation, College Station, TX).

RESULTS

Patient Characteristics

The characteristics of the subjects are shown in Table 1. A total of 473 patients who were enrolled between 1995 and 2008 in an ongoing prospective cohort study and who underwent curative therapy for early stage NSCLC at MD Anderson Cancer Center were included. Adenocarcinoma was the most common histology involving 61.5% of patients. A majority of patients (73.3%) underwent surgical resection only and the remaining 26.7% of patients received either neoadjuvant or adjuvant chemotherapy. Median follow-up time was 61 months.

Association of Telomere Length with Recurrence and Other Clinicopathologic Parameters

Relative telomere length (RTL) results from a total of 473 peripheral blood samples that were collected at time of in-person interview were used for the analysis. Age and RTL showed inverse association ($\theta = -0.00807774$; $p = 1.50X10^{-8}$). At the time of analysis, 151 patients (32%) developed recurrence. Our results show that recurrence group demonstrated significantly longer mean RTL compared with nonrecurrence group (1.13 versus 1.07, $p = 0.0465$) (Table 2 and Fig. 1). In addition, women had longer RTL compared with men, and the patients with adenocarcinoma demonstrated longer RTL compared with those with other histologic types (Table 2 and Fig. 1). There was no significant association of RTL with smoking status, ethnicity, stage of disease, and type of

