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Prognostic Significance of Ataxia-Telangiectasia Mutated, DNA-dependent Protein Kinase Catalytic Subunit, and Ku Heterodimeric Regulatory Complex 86-kD Subunit Expression in Patients With Nonsmall Cell Lung Cancer

Jinliang Xing, MD, PhD¹, Xifeng Wu, MD, PhD¹, Ara A. Vaporciyan, MD², Margaret R. Spitz, MD, MPH¹, and Jian Gu, PhD¹

¹Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

²Department of Thoracic and Cardiovascular Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Abstract

BACKGROUND—The double-strand break (DSB) repair capacity has been implicated in the survival of patients in several cancer types. However, little is known about the prognostic importance of the key DSB repair genes—ataxia-telangiectasia mutated (*ATM*), DNA-dependent protein kinase catalytic subunit (*DNA-PKcs*), and the Ku heterodimeric regulatory complex 86-kD subunit (*Ku80*)—in nonsmall cell lung cancer (NSCLC). To address this issue, the authors determined the messenger RNA (mRNA) expression of these genes in patients NSCLC and assessed their prognostic relevance.

METHODS—mRNA expression levels of *ATM*, *DNA-PKcs*, and *Ku80* were measured in tumor and adjacent normal tissues from 140 patients with NSCLC by using quantitative real-time polymerase chain reaction analysis. Then, a Cox proportional hazards regression model and Kaplan-Meier plots were used to evaluate the association between the tumor:normal (T/N) expression ratios of the 3 genes and the overall survival rate and duration in patients with NSCLC.

RESULTS—mRNA expression of *ATM* and *DNA-PKcs*, but not of *Ku80*, was significantly higher in tumor tissues than in adjacent normal tissues ($P = .003$ and $P < .001$, respectively). The high T/N expression ratios of *ATM* and *DNA-PKcs* were associated significantly with a 1.82-fold increased risk of death (95% confidence interval, 1.05–2.70) and a 2.13-fold increased risk of death (95% confidence interval, 1.21–3.76), respectively. However, no significant association with risk was observed for *Ku80*. Kaplan-Meier analyses revealed that patients with high T/N expression ratios of *ATM* or *DNA-PKcs* had notably shorter median survival than patients with low ratios.

CONCLUSIONS—The current findings suggested that the T/N expression ratios of *ATM* and *DNA-PKcs* may be useful for identifying NSCLC patients with a poor prognosis who may benefit from more aggressive therapy.

Keywords

DNA repair; DNA double-strand break; nonsmall cell lung cancer; prognosis

Lung cancer is the leading cause of cancer death in both men and women in the United States with an estimated 213,380 new cancer cases and 160,390 deaths in 2007.¹ The current 5-year survival rates are <15%, a rate that has improved little over the past 2 decades.² Recently, efforts have been made to identify new prognostic markers for lung cancer on the basis of biologic characteristics, such as gene expression profiles and specific molecular abnormalities. These markers may allow clinicians to identify appropriate candidates for specific therapies, thus improving the outcome of patients with lung cancer.

DNA double-strand breaks (DSBs) are regarded as the most lethal of all DNA lesions.³ Prompt and efficient repair of DNA DSBs is critical for maintaining genomic integrity. Defects in the DSB repair pathway may cause genetic alterations, chromosome instability, and, ultimately, malignant transformation.^{4,5} Conversely, tumor cells with increased DNA DSB repair capacity would be more likely to survive and proliferate, leading to the poor prognosis in cancer patients. Thus, the messenger RNA (mRNA) expression levels of key genes in the DSB repair pathway may be used as prognostic markers for cancer patients.

The ataxia-telangiectasia mutated (ATM) protein has been identified as the principal activator and master controller of cellular response to DSBs. ATM phosphorylates the key genes of the DNA damage response network and, thus, initiates cell cycle arrest, apoptosis, and DNA repair.^{6,7} Individuals with inherited mutations or polymorphisms in the ATM gene (*ATM*) are more susceptible to various cancers, including lung cancer, breast cancer, leukemia, and lymphoma.⁸⁻¹¹ In addition, altered *ATM* expression levels have been identified in colon and pancreatic tumor tissues.^{12,13} Accordingly, the prognostic value of *ATM* expression in colorectal and breast cancer has been investigated.^{12,14} However, to our knowledge, its significance in lung cancer has not been studied.

The nonhomologous end-joining (NHEJ) pathway is the dominant mechanism for the repair of DSBs in mammalian cells. A key element of the NHEJ pathway is the DNA-dependent protein kinase (DNA-PK), which consists of a 465-kD catalytic subunit, DNA-PKcs, and a heterodimeric regulatory complex, Ku, which includes a 70-kD subunit (Ku70) and a 86-kD subunit (Ku80).¹⁵ It has been reported that cells lacking DNA-PKcs or Ku80 have defective DNA DSB repair.^{16,17} Recent reports^{10,18} suggest that a positive relation exists between Ku and cancer development. Higher levels of Ku or DNA-PKcs expression have been identified in bladder,¹⁹ colon,²⁰ nasopharyngeal,²¹ and cervical²² tumor tissues compared with corresponding normal tissues. The expression patterns of Ku and DNA-PKcs also reportedly have been associated with tumor radiosensitivity and survival in patients with rectal,²³ nasopharynx,²¹ and cervical²² carcinomas. Nonetheless, to our knowledge, no similar analyses of Ku80 and DNA-PKcs expression have been conducted to date in patients with nonsmall cell lung cancer (NSCLC).

In this study, we determined the mRNA expression levels of the *ATM*, *DNA-PKcs*, and *Ku80* genes in tissue samples from 140 patients with NSCLC and compared those levels with patients' overall survival durations and rates to determine their prognostic value. To our knowledge, this is the first study of the association between *ATM*, *DNA-PKcs*, and *Ku80* mRNA expression and survival in patients with NSCLC.

MATERIALS AND METHODS

Patients and Tissue Samples

In total, 140 patients were recruited for this study, all of whom had histologically confirmed NSCLC and underwent curative surgery at the University of Texas M. D. Anderson Cancer Center in Houston, Texas (M. D. Anderson) between 1993 and 1997. There were no age,

sex, ethnicity, or tumor stage restrictions on patient enrollment. All patients gave written informed consent, and the study protocol was approved by the M. D. Anderson Institutional Review Board. Patients' demographic variables and smoking history were obtained by chart review. Clinical data were collected from the computerized tumor registry at M. D. Anderson. Patients were followed until June 2005. Tissue samples (tumor and adjacent normal tissues) were obtained and snap-frozen in liquid nitrogen immediately after excision and stored at -80°C until further use. The World Health Organization classification system was used for histopathologic typing and grading of tumors, and the 1997 revisions of the International System for Staging Lung Cancer guidelines were used for staging.²⁴

RNA Extraction and Complementary DNA Synthesis

Total RNA was extracted from frozen tissues by using the EZNA RNA kit (Omega Bio-tek, Doraville, Ga) in accordance with the manufacturer's protocol. The RNA concentration was measured by using a spectro-photometer (Beckman Coulter, Fullerton, Calif). The quality of RNA samples was determined by 1% agarose gel electrophoresis and ethidium bromide staining. Then, the complementary DNA (cDNA) was synthesized by using a reverse transcription kit (Applied Biosystems, Branchburg, NJ) in a final volume of 20 μL containing 0.5 μg of total RNA, 5 mM MgCl_2 , 250 μM of each deoxyribonucleotide triphosphate (dNTP), 20 U of RNase inhibitor, 50 U of multiscribe reverse transcriptase (RT), 2.5 μM random hexamers, and 1 $\times$ RT buffer. The reaction was carried out under the following thermal cyclers conditions: 10 minutes at 25°C , 30 minutes at 42°C , and 5 minutes at 99°C . All cDNA products were stored at -30°C until they were used in the real-time polymerase chain reaction (PCR) analysis.

Real-time PCR

The primers and probes that were used for real-time PCR were designed by using Primer Express software (version 2.0; Applied Biosystems). We searched the Genbank database to confirm the specificity of primers and probes and the absence of single nucleotide polymorphisms. To avoid amplifying contaminated genomic DNA, 1 of the 2 primers was designed to span 2 exons. The sequences of all primers and probes are listed in Table 1. Real-time PCR was performed with a 384-well optic plate on the ABI Prism 7900 sequence detection system (Applied Biosystems). The PCR was carried out in a total volume of 10 μL , which consisted of 1 $\times$ Taqman buffer A, 3.4 mM MgCl_2 , 100 μM of each dNTP, 0.2 μM of each primer, 0.1 μM probe, and 0.02 U of AmpliTaq Gold DNA polymerase. The following reaction conditions were used: 10 minutes at 95°C to activate the Ampli-Taq Gold enzyme, then 15 seconds at 95°C and 1 minute at 60°C for 40 cycles. For each tested gene, the standard curve was generated by using commercial human total RNA (Stratagene, La Jolla, Calif) to quantify each transcript in unknown samples. Three housekeeping genes, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), 18S ribosomal RNA (*18S rRNA*), and β -*actin*, were used as endogenous controls to normalize the RNA input amount as well as the reverse transcription efficiency and RNA quality on the basis of the geometric mean values of their mRNA expression. The PCR reaction for each sample was performed in duplicate to determine the consistency of the results.

Statistical Analysis

At the end of the study, 2 patients had recurrences only, 37 patients had metastasis only, and 4 patients had both recurrences and metastasis. Because the assessment of time to recurrence or metastasis after surgery in patients with NSCLC is less reliable and less precise than that of overall survival, we only evaluated overall survival as an endpoint in this study. The overall survival duration was defined as the time from lung cancer diagnosis to patient death or last follow-up. Smoking status was categorized as 1 of the following: never-smoker, an individual who had smoked fewer than 100 cigarettes in his or her lifetime; former smoker,

an individual who had stopped smoking ≥ 1 year before being diagnosed with cancer; and current smoker, an individual who continued smoking or who had stopped smoking <1 year before being diagnosed with cancer. Pack-years were calculated as the mean number of cigarettes smoked per day divided by 20 and then multiplied by years of smoking. We evaluated the distribution of patient characteristics by survival status (alive or dead) using the Pearson chi-square test or the Fisher exact test for categorical variables and the Student *t* test for continuous variables. The difference in mRNA expression level between tumor and adjacent normal tissues was compared by using the Wilcoxon rank-sum test. The Spearman correlation coefficient test was used to assess the correlation between the mRNA expression of *ATM* and *DNA-PKcs*, *ATM* and *Ku80*, and *Ku80* and *DNA-PKcs* in both tumor tissues and normal tissues. The tumor:normal (T/N) expression ratios of all 3 genes were dichotomized as high or low, and the median T/N expression values were used as the cutoff points. A Cox proportional-hazards regression model was used to determine the hazard ratios (HRs) and 95% confidence intervals (95% CIs) and was adjusted for age, sex, ethnicity, smoking status, tumor grade, clinical disease stage, and treatment. The association between the overall survival duration and the T/N expression ratios of the 3 genes was evaluated by using Kaplan-Meier plots and the log-rank test. All analyses were performed by using STATA statistical software (version 8.0; STATA Corp., College Station, Tex). All statistical tests were 2-sided, and *P* values $<.05$ were considered statistically significant.

RESULTS

Patients' Clinical and Tumor Characteristics

One hundred forty patients with NSCLC (mean age, 65 years) were included in this study (Table 2), including 133 white patients (95%) and 122 ever-smokers (former smokers plus current smokers; 87%). Seventy-four patients (53%) had adenocarcinoma, and 36 patients (26%) had squamous cell carcinoma. Sixty-six patients (47%) had stage I disease, 23 patients (16%) had stage II disease, and 33 patients (24%) had stage III disease. The degree of differentiation was moderate (grade 2) in 55 patients (39%) and poor (grade 3) in 56 patients (40%). Fifty-eight patients (41%) survived for ≥ 5 years (median survival for all patients, 39.7 months), and 44 patients (31%) remained alive at the end of the study period. High tumor stage and grade at diagnosis, as expected, were associated significantly with a higher risk of death ($P = .02$ and $P = .03$, respectively). Among 130 patients with complete treatment information, 71 patients (55%) underwent surgery alone, and 59 patients (45%) underwent surgery and received adjuvant chemotherapy or radiotherapy. Compared with patients who underwent surgery alone, patients who underwent surgery and received adjuvant therapy had a significantly higher death rate ($P < .001$); mainly because adjuvant therapy as a secondary treatment was received mostly by patients who had high-stage disease and were at high risk of recurrence and metastasis. No significant differences were observed in the distribution of patients according to age ($P = .37$), sex ($P = .50$), ethnicity ($P = .58$), smoking status ($P = .34$), pack-years ($P = .77$), or tumor histologic subtype ($P = .81$) between the patients who remained alive and the patients who died (Table 2).

Messenger RNA Expression of *ATM*, *DNA-PKcs*, and *Ku80* in Tumor and Normal Tissues

The median mRNA expression levels were significantly higher in tumor tissues than in adjacent normal tissues for *ATM* (0.080 vs 0.058; $P = .003$) and *DNA-PKcs* (0.073 vs 0.051; $P < .001$) but not for *Ku80* (0.256 vs 0.222; $P = .491$) (Table 3). The mRNA expression levels of *ATM*, *DNA-PKcs*, and *Ku80* were correlated with one another in both tumor tissues and adjacent normal tissues. The correlation coefficients for *ATM* and *DNA-PKcs*, *ATM* and *Ku80*, and *Ku80* and *DNA-PKcs* were 0.71 ($P < .001$), 0.80 ($P < .001$), and 0.72 ($P < .001$), respectively, in tumor tissues and 0.64 ($P < .001$), 0.66 ($P < .001$), and 0.51 ($P < .001$), respectively, in normal tissues (Table 4). The mRNA expression levels of *ATM*,

DNA-PKcs, and *Ku80* in tumor tissues were not associated significantly with age, sex, tumor stage, grade, or histologic type (data not shown).

Association Between T/N Expression Ratios of *ATM*, *DNA-PKcs*, and *Ku80* and Overall Survival

We dichotomized the T/N expression level of *ATM*, *DNA-PKcs*, and *Ku80* into high and low groups by using the median value as the cutoff point. After adjustment for age, sex, ethnicity, smoking status, tumor stage, clinical disease stage, and treatment, the multivariate Cox proportional-hazards model revealed that the T/N expression ratios of *ATM* and *DNA-PKcs* were associated significantly with patients' overall survival rates (Table 5). Patients who had high T/N expression ratios of *ATM* and *DNA-PKcs* had 1.82-fold (95% CI, 1.05–2.70; $P = .032$) and 2.13-fold (95% CI, 1.21–3.76; $P = .009$) increased risks of death, respectively, compared with patients who had low T/N expression ratios. No statistically significant association was observed between the T/N expression ratio of *Ku80* and overall survival (HR, 1.32; 95% CI, 0.79–2.21; $P = .283$).

Kaplan-Meier analysis revealed that patients who had high T/N expression ratios of *DNA-PKcs* had significantly shorter median survival time (MST) than patients who had low T/N expression ratios (34.8 months vs 64.9 months; $P = .013$; log-rank test) (Fig. 1). Similar results were obtained for patients who had high and low T/N expression ratios of *ATM* (MST: 37.8 months vs 60.9 months; $P = .137$, log-rank test), although the difference did not reach statistical significance. The MST for patients who had high and low expression ratios of *Ku80* was 38.83 and 47.63 months, respectively (log-rank test; $P = .70$).

Stratified analyses revealed associations between the T/N expression ratio of *ATM* and the risk of death in younger patients (aged <66 years; HR, 3.71; 95% CI, 1.29–10.72; $P = .015$) but not in older patients (aged ≥ 66 years; HR, 1.07; 95% CI, 0.48–2.37; $P = .875$), in women (HR, 3.79; 95% CI, 1.16–12.39; $P = .027$) but not in men (HR, 1.05; 95% CI, 0.47–2.38; $P = .901$), and in light smokers (HR, 8.04; 95% CI, 2.09–30.96; $P = .002$) but not in heavy smokers (HR, 1.36; 95% CI, 0.63–2.96; $P = .434$) (Table 5). Similarly, high T/N expression ratios of *DNA-PKcs* also were associated significantly with a low survival rate in younger patients (aged <66 years; HR, 3.75; 95% CI, 1.35–10.47; $P = .011$) but not in older patients (aged ≥ 66 years; HR, 1.71; 95% CI, 0.80–3.65; $P = .164$), in women (HR, 4.78; 95% CI, 1.59–14.41; $P = .005$) but not in men (HR, 1.70; 95% CI, 0.75–3.88; $P = .207$), and in light smokers (HR, 3.24; 95% CI, 1.17–8.98; $P = .024$) but not in heavy smokers (HR, 2.09; 95% CI, 0.89–4.92; $P = .092$). In addition, the risk of death conferred by high T/N ratios appeared to be evident in patients with adenocarcinoma but not in patients with squamous cell carcinoma for both *ATM* and *DNA-PKcs*. We also performed stratified analysis by adjuvant therapy and observed that the association between the T/N expression ratio of *ATM* and the risk of death was evident in patients who underwent surgery alone (HR, 2.71; 95% CI, 1.11–6.64; $P = .029$) but not in patients who underwent surgery and received adjuvant therapy (HR, 1.00; 95% CI, 0.47–2.10; $P = .989$), whereas the association for *DNA-PKcs* was stronger in patients who underwent surgery and received adjuvant therapy (HR, 2.52; 95% CI, 1.13–5.65; $P = .024$) than in patients who underwent surgery alone (HR, 1.78; 95% CI, 0.80–3.97; $P = .158$). No significant association was observed between the T/N expression ratio of *Ku80* and the risk of death for either treatment group.

DISCUSSION

In the current study, we determined the mRNA expression of 3 key components of the DNA DSB repair pathway in patients with NSCLC and observed significantly higher expression in tumor tissues compared with normal tissues for *ATM* and *DNA-PKcs* but not *Ku80*. The high T/N expression ratios of *ATM* and *DNA-PKcs*, but not *Ku80*, were associated

significantly with increased risks of death and notably shorter MST compared with the low T/N expression ratios. To our knowledge, this is the first study to measure mRNA expression of these 3 genes in NSCLC tissue and to report that *ATM* and *DNA-PKcs* may be useful prognostic markers in NSCLC.

There have been some studies evaluating the expression of these genes in other cancer tissues. Consistent with our results, Hosoi et al.²⁰ observed an elevated mRNA levels of *DNA-PKcs* in colorectal cancer tumor tissues compared with normal tissues. Studies of *ATM* and *Ku80* expression in tumor tissues have yielded inconsistent findings across different cancer types, with some reporting higher expression, some reporting lower expression, and others reporting no changes.^{12,19,20,25-27} The inconsistency in *ATM* and *Ku80* regulation in different tumor types suggests a remarkable difference on the molecular alterations related to the progression of cancer at different sites. In addition, the methodology of measuring mRNA expression and the selection of control genes also may contribute to the inconsistency of results. In this study, we used the geometric mean of the expression of 3 housekeeping genes (*GAPDH*, *18S rRNA*, and β -*actin*) as internal controls to normalize target gene expression. Vandesompele et al.²⁸ provided compelling evidence to support the finding that geometric averaging of multiple internal control genes offers more accurate normalization of real-time quantitative RT-PCR data than single internal control genes. This method can eliminate the expression variability of a single control gene in response to various factors. In the current study, we also compared the use of single and multiple housekeeping genes and observed that the variations were much smaller when the data were normalized to the geometric mean of 3 housekeeping genes than any individual gene (data not shown). Most previous studies used a single gene as an internal control; therefore, it is difficult to compare the results across different studies.

The mechanisms of *ATM*, *DNA-PKcs*, and *Ku80* regulation remain to be elucidated. However, exogenous and endogenous factors may play a role in their regulations. Jiang et al.²⁵ observed that the benzo (a)pyrene diol epoxide in tobacco smoke was responsible for increased *ATM* expression levels in premalignant and malignant esophageal tissues, and Hosoi et al.²⁰ demonstrated that overexpression of the transcription factor SP1 contributes to elevated levels of *DNA-PKcs* and *Ku70/Ku80*. A correlation between *Ku80* and *DNA-PKcs* expression has been reported in patients with colorectal cancer and head and neck cancer.^{20,29} The promoter regions of both *Ku80* and *DNA-PKcs* contain consensus SP1 recognition elements; thus, *DNA-PKcs* and *Ku80* may be regulated transcriptionally by SP1. A connection also may exist between the levels of *DNA-PKcs* and *ATM*.³⁰ Peng et al.³¹ demonstrated that the amount or activity of *DNA-PKcs* influenced *ATM* expression. In our study, the mRNA levels of *ATM*, *DNA-PKcs*, and *Ku80* were correlated with one another. These findings suggest that there are highly coordinated efforts of the cellular defense system in repairing DNA DSBs.

Most noteworthy, we observed that high expression of *ATM* and *DNA-PKcs* was associated with poor overall survival in patients with NSCLC. Safar et al.³² reported that *ATM* hypermethylation was associated with good overall survival in patients with NSCLC, and Bosken et al.³³ observed that patients with NSCLC who had effective systemic nucleotide excision repair had poorer survival than patients who had suboptimal nucleotide excision repair. Those results are consistent with our findings. However, there also have been some inconsistent results in terms of the prognostic value of these 3 genes in other cancer types. For example, low *ATM* expression has been associated with an increased risk of death in colorectal and breast cancers.^{12,14} Again, these inconsistencies may be the results of many factors, including different cancer types, different histology and tumor characteristics, and accuracy in measuring mRNA expression. In addition, all of the literature on the prognostic value of *ATM*, *DNA-PKcs*, and *Ku80* discussed above were based on gene expression in

tumor tissues. However, in our study, we attempted to assess the possibility of using the T/N expression ratios of 3 DNA repair genes as prognostic markers. We compared the prognostic values between T/N expression ratios and tumor expression levels of the 3 genes by using a multivariate Cox proportional-hazards model. Patients with a high T/N expression ratios of *ATM* and *DNA-PKcs* exhibited significantly increased risk of death, as discussed above. However, no significant associations were observed between survival and tumor expression levels of *ATM* (HR, 0.92; 95% CI, 0.53–1.60; $P = .766$), *Ku80* (HR, 1.48; 95% CI, 0.90–2.43; $P = .120$), or *DNA-PKcs* (HR, 1.17; 95% CI, 0.71–1.92; $P = .530$). Therefore, we believe that, for patients with cancer, the T/N expression ratio, which reflects alteration of the gene expression level, may be a better predictor of prognosis, although the requirement of having normal tissue available for analysis is a limitation for measurement of the T/N expression ratio.

The molecular mechanism of cell survival modulation by *ATM* and *DNA-PKcs* remains to be elucidated. Harima et al.²² suggested that tumor cells with a greater ability to repair damaged DNA are more likely to survive and proliferate. In a comprehensive review, Kaina also reported that cells that exhibit a defect in various DNA repair pathways are liable to suffer from DNA damage-triggered apoptosis.³⁴ Therefore, we speculate that high expression of *ATM* and *DNA-PKcs* leads to increased DNA DSB repair capacity in tumor cells and that tumor cells with higher DSB repair capacity would be more likely to survive, proliferate, and metastasize. In addition, the expression of *ATM* modulates patients' survival by triggering growth factor-mediated pathways³⁵; for example, *ATM* may increase the transcriptional expression of the insulin-like growth factor-1 receptor, a cell surface molecule with tyrosine kinase activity that can mediate mitogenesis, cell transformation, and apoptosis inhibition. Other functional roles of *ATM* and *DNA-PKcs* in survival modulation are under investigation.

In the current study, no significant correlation was observed between the mRNA expression of *ATM*, *Ku80*, and *DNA-PKcs* in tumor tissues and patients' age, sex, tumor grade, stage, and histologic type, further suggesting that the effect of the 3 DNA repair genes on patient survival is independent of these clinical characteristics. In addition, the results of our stratified analyses indicate that the prognostic values of *ATM* and *DNA-PKcs* are significant only in younger patients (aged <66 years), in women, in light smokers, and in patients with adenocarcinoma, suggesting that age, sex, smoking, and histologic type may play critical roles in modifying the prognostic importance of *ATM* and *DNA-PKcs* expression in patients with NSCLC. In addition, adjuvant therapy also appeared to have different modifying effects on the prognostic value of *ATM* and *DNA-PKcs* expression. This result may have been obtained because of the different functions of *ATM* and *DNA-PKcs* in DNA repair and cell proliferation. However, the results from these stratified analyses should be interpreted with caution given the small sample size in each stratum. Large studies are warranted to confirm these findings.

In summary, we observed that the expression levels of *ATM* and *DNA-PKcs* were higher in NSCLC tumor tissues than in normal tissues and that high T/N expression ratios of *ATM* and *DNA-PKcs* were associated significantly with poor survival in patients with NSCLC. The current results indicate that *ATM* and *DNA-PKcs* may be useful in predicting the clinical outcome of patients with NSCLC, thus helping physicians to determine optimal therapies for individual patients.

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FIGURE 1.

Kaplan-Meier survival estimates for all patients. Survival duration according to the expression of ataxia-telangiectasia mutated (*ATM*) (A) and DNA-dependent protein kinase catalytic subunit (*DNA-PKcs*) (B). MST indicates median survival time.

TABLE 1
Oligonucleotide Sequence of Primers and Probes for Real-time Polymerase Chain Reaction Analysis

Gene	Sequence (5'-3')
<i>ATM</i>	
Forward primer	TTATTTACTGGGTCAGCCTGCA
Reverse primer	GAAC TATACTGGTGGTCAGTGCCA
Probe	FAM-ACCTTCATGTCCTGCAGTATGCTGTTTGA CT-TAMRA
<i>DNA-PKcs</i>	
Forward primer	CGTGTTTATCAGATGCCAAGCT
Reverse primer	CACCTCAGGAACTGTGTCAAGG
Probe	FAM-TCCAGTCTGTTGCAAGCGTCTTGCTGT-TAMRA
<i>Ku80</i>	
Forward primer	GGAGCCCACTTCAGCGTCT
Reverse primer	CAGCAGGATTCACACTTCCAAC
Probe	FAM-CAGTCTGGCTGAAGGCAGTGTCACCTC-TAMRA
<i>GAPDH</i>	
Forward primer	AAGGCTGAGAACGGAAGC
Reverse primer	GAGGGATCTCGTCCTGGA
Probe	FAM-TGTCATCAATGGAAATCCCATCACCATC-TAMRA
<i>18S rRNA</i>	
Forward primer	AGTCCCTGCCCTTTGTACACA
Reverse primer	GATCCGAGGGCCTCACTAAAC
Probe	FAM-CGCCCCTCGCTACTACCGATTGG-TAMRA
<i>β-actin</i>	
Forward primer	CGAGCGCGGCTACAGCTT
Reverse primer	TCCTTAATGTCACGCACGATTT
Probe	FAM-ACCACCACGGCCGAGCGG-TAMRA

ATM indicates ataxia-telangiectasia mutated; T, thymine; A, adenine; C, cytosine; G, guanine; FAM, 6-carboxyfluorescein probe; TAMRA, 6-carboxytetramethyl-rhodamine probe; *DNA-PKcs*, DNA-dependent protein kinase catalytic subunit; *Ku80*, the Ku heterodimeric regulatory complex 86-kD subunit; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; rRNA, ribosomal RNA.

TABLE 2
Distribution of Demographic and Clinical Variables by Survival Status

Variable	No. of patients (%)		P
	Alive (n = 44)	Dead (n = 96)	
Sex			
Men	23 (52)	56 (58)	.502
Women	21 (48)	40 (42)	
Ethnicity			
White	42 (96)	91 (95)	.580
Hispanic	0 (0)	2 (2)	
African American	2 (5)	3 (3)	
Smoking status *			
Never	6 (14)	6 (7)	.341
Former	16 (38)	37 (40)	
Current	20 (48)	49 (53)	
Histologic type			
Adenocarcinoma	23 (52)	51 (53)	.812
Squamous cell carcinoma	12 (27)	24 (25)	
Other	9 (20)	21 (22)	
Tumor stage *			
I	26 (70)	40 (47)	.033
II	7 (19)	16 (19)	
III	4 (11)	29 (34)	
Grade *			
1	7 (20)	4 (5)	.027
2	14 (40)	41 (47)	
3	14 (40)	42 (48)	
Treatment *			
Surgery alone	31 (44)	40 (56)	<.001
Surgery with adjuvant therapy	8 (14)	51 (86)	
Mean age $\pm$ SD, y	63.9 $\pm$ 10.6	65.7 $\pm$ 10.9	.367
Mean pack-y $\pm$ SD [†]	51.2 $\pm$ 32.2	52.7 $\pm$ 25.1	.774

SD indicates standard deviation.

* Information on smoking status, tumor stage, tumor grade, and adjuvant treatment was unavailable for a few patients.

[†] For ever-smokers only

TABLE 3
Expression of DNA Double-strand Break Repair-related Genes in Tumor and Normal Tissues

Gene	Normal tissue		Tumor tissue		P
	No. of patients*	Median (95% Range) [†]	No. of patients*	Median (95% Range) [†]	
<i>ATM</i>	122	0.058 (0.003–1.394)	131	0.080 (0.003–1.216)	.003
<i>Ku80</i>	127	0.222 (0.035–1.856)	140	0.256 (0.021–1.220)	.491
<i>DNA-PKcs</i>	127	0.051 (0.010–0.663)	140	0.073 (0.015–1.237)	<.001

ATM indicates ataxia-telangiectasia mutated; *Ku80* the Ku heterodimeric regulatory complex 86-kD subunit; *DNA-PKcs*, DNA-dependent protein kinase catalytic subunit.

*Numbers of patients may not add up to 140 because of insufficient tissue samples or assay failure.

[†]The 95% range was defined as the 2.5th percentile to the 97.5th percentile.

TABLE 4
Associations Among Messenger RNA Expression Levels of Ataxia-telangiectasia Mutated, the Ku Heterodimeric Regulatory Complex 86-kD Subunit, and the DNA-dependent Protein Kinase Catalytic Subunit in Tumor and Normal Tissues

mRNA expression	Tumor tissue					Normal tissue				
	ATM		Ku80			DNA-PKcs		ATM		
	CC	P	CC	P	CC	CC	P	CC	P	CC
ATM			0.80	<.001	0.71	<.001		0.66	<.001	0.56
Ku80	0.80	<.001			0.70	<.001	0.66	<.001		0.51
DNA-PKcs	0.71	<.001	0.70	<.001			0.56	<.001	0.51	<.001

ATM indicates ataxia-telangiectasia mutated; Ku80, the Ku heterodimeric regulatory complex 86-kD subunit; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; mRNA, messenger RNA; CC, correlation coefficient

TABLE 5
Association Between Messenger RNA Expression of DNA Repair-related Genes in Tumor and Normal Tissues and Survival in Patients With Nonsmall Cell Lung Carcinoma

mRNA expression (T/N)*	ATM		Ku80		DNA-PKcs	
	HR (95% CI)†	P	HR (95% CI)	P	HR (95% CI)†	P
Overall	1.82 (1.05–2.70)‡	.032	1.32 (0.79–2.21)	.283	2.13 (1.21–3.76)‡	.009
Age, y§						
<66	3.71 (1.29–10.72)‡	.015	1.52 (0.64–3.63)	.345	3.75 (1.35–10.47)‡	.012
66	1.07 (0.48–2.37)	.875	1.01 (0.48–2.14)	.974	1.71 (0.80–3.65)	.164
Sex						
Men	1.05 (0.47–2.38)	.901	1.09 (0.52–2.26)	.823	1.70 (0.75–3.88)	.207
Women	3.79 (1.16–12.39)‡	.027	2.16 (0.90–5.16)	.083	4.78 (1.59–14.41)‡	.005
Smoking status						
Never	—	—	—	—	—	—
Ever	1.53 (0.86–2.73)	.150	1.32 (0.77–2.26)	.310	1.93 (1.11–3.88)‡	.021
Pack-y						
<50	8.04 (2.09–30.96)‡	.002	2.05 (0.76–5.51)	.156	3.24 (1.17–8.98)‡	.024
50	1.36 (0.63–2.96)	.434	1.37 (0.65–2.91)	.408	2.09 (0.89–4.92)	.092
Histologic type						
Adenocarcinoma	1.99 (0.97–4.06)	.060	1.18 (0.64–2.17)	.593	1.90 (0.99–3.62)	.052
Squamous cell carcinoma	1.66 (0.60–4.59)	.329	1.38 (0.47–4.11)	.560	1.90 (0.58–6.25)	.291
Treatment						
Surgery alone	2.71 (1.11–6.64)‡	.029	1.99 (0.84–4.70)	.116	1.78 (0.80–3.97)	.158
Surgery with adjuvant therapy	1.00 (0.47–2.10)	.989	0.75 (0.37–1.53)	.436	2.52 (1.13–5.65)‡	.024‡

ATM indicates ataxia-telangiectasia mutated; Ku80, the Ku heterodimeric regulatory complex 86-kD subunit; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; T/N, the ratio of gene expression in tumor tissue to gene expression in normal tissue; HR, hazard ratio; CI, confidence interval.

*Dichotomized by using the median value for messenger RNA expression (T/N). The cutoff points for ATM, Ku80, and DNA-PKcs were 1.744, 1.142, and 1.670, respectively.

†Adjusted for age, sex, ethnicity, smoking status, tumor grade, clinical disease stage, and treatment as appropriate.

‡Significant associations ($P < .05$).

[§]Dichotomized by using the median value in all patients.
^{//}Dichotomized by using the median value in ever smokers.