TERT Mutations in Non–Small Cell Lung Cancer: Clinicopathologic Features and Prognostic Implications

Liu Yang[1,2*] (ID), Meng Wang[1*], Na Li[1], Lu-Da Yan[1], Wen Zhou[1], Zhi-Qiong Yu[1], Xiao-Chun Peng[3,4*], Jun Cai[1*] and Yong-Hua Yang[1*]

[1]Department of Oncology, First Affiliated Hospital of Yangtze University, Jingzhou, China. [2]Department of Oncology, Gong An County People's Hospital, Jingzhou, China. [3]Laboratory of Oncology, Center for Molecular Medicine, School of Basic Medicine, Health Science Center, Yangtze University, Jingzhou, China. [4]Department of Pathophysiology, School of Basic Medicine, Health Science Center, Yangtze University, Jingzhou, China.

ABSTRACT

INTRODUCTION: The associations between the clinical characteristics of non–small cell lung cancer (NSCLC) and mutations in *telomerase reverse transcriptase* (*TERT*) gene remain unclear. In this study, we used next-generation sequencing (NGS) to investigate the incidence rate and clinical correlates of TERT mutations in patients with NSCLC.

METHODS: In total, 283 tumor samples from patients with NSCLC were tested using an NGS panel from September 2017 to May 2020. The genetic testing results and clinical data of all patients were collected.

RESULTS: TERT mutations were found in 30 patients, which were significantly associated with age, smoking history, sex, and metastasis ($P < 0.05$). Survival analyses showed that patients who carried *TERT* mutations had a poorer prognosis. Of the 30 *TERT*-mutation carriers, 17 harbored *epidermal growth factor receptor* (*EGFR*) mutations, which were significantly associated with sex, histopathology type, and metastasis ($P < 0.05$; overall survival [OS], 21 months; 95% confidence interval [CI], 8.153-33.847 months). Three *TERT* mutation patients harbored *Kirsten rat sarcoma virus* (*KRAS*) mutations, which were significantly associated with metastasis risk ($P < 0.05$), *KRAS* mutations carriers had a worse prognosis, with an OS of 10 months (95% CI, 8.153-33.847 months). Multivariate Cox regression analyses showed that age, cancer stage, and *TERT* mutation carrier status were independent risk factors for NSCLC, and the *TERT* mutation was 2.731 times higher than that without *TERT* mutation (95% CI, 1.689-4.418, $P < 0.001$).

CONCLUSIONS: *TERT* mutations were present in 11% of patients with NSCLC. *TERT* mutations were associated with age, smoking history, sex, and distant metastasis. Co-mutations in *TERT* and *EGFR/KRAS* indicated a poor prognosis. The co-mutations of *TERT* and *EGFR* differed according to sex, histopathology type, and metastasis, whereas *TERT* and *KRAS* co-mutations were only associated with patient metastasis. Age, cancer stage, and *TERT* mutation carrier status were independent risk factors for poor prognosis in patients with NSCLC.

KEYWORDS: Non–small cell lung cancer, TERT, co-mutation, prognosis

Introduction

Lung cancer is the second most common cause of morbidity worldwide and the leading cause of cancer-related death worldwide for both sexes.[1] According to the histological classification, 85% of lung cancers are non–small cell lung cancers (NSCLCs).[2]

It is estimated that up to 69% of patients with advanced NSCLC have treatable mutations in multiple genes, *including epidermal growth factor receptor* (*EGFR*), *Kirsten rat sarcoma virus* (*KRAS*), *anaplastic lymphoma kinase* (*ALK*), *mesenchymal–epithelial transition factor receptor* (*MET*), *c–ROS oncogene 1* (*ROS1*), *human epidermal growth factor receptor* (*HER2*), and

V–RAF mouse sarcoma oncogene homolog B1 (*BRAF*).[3] *Telomerase reverse transcriptase* (*TERT*) mutations have been detected in various tumors, including lung cancer. However, mutation rates vary among studies.[4-6]

Telomerase is a key enzyme for cell survival, and the basis of tumorigenesis is the infinite proliferation of malignant cells, which in most cases is achieved by activating telomerase.[7] Inhibition and/or shortening of telomerase in human cells is considered a natural evolutionary strategy against cancer,[8] and telomerase activation and telomere damage are related to the occurrence of human lung cancer.[9,10]

TERT is the main factor that controls telomerase activity.[11] *TERT* point mutation, amplification, rearrangement, and epigenetic modification through the *TERT* promoter can regulate

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*Liu Yang, Meng Wang, Xiao-Chun Peng, Jun Cai and Yong-Hua Yang have contributed equally to this work and share first authorship.

Table 1. Demographics and clinicopathologic features of 283 patients with non–small cell lung cancer.

CLINICAL CHARACTERISTICS	NO. OF PATIENTS	*TERT* WILD-TYPE	*TERT* MUTATION	*P* VALUE
Total	283	253	30	
Sex				<.0001
Male	164(58%)	147	17	
Female	119(42%)	106	13	
Age				<.0001
<60	95(34%)	84	11	
≧60	188(66%)	169	19	
Smoking history				<.0001
Smokers	105(37%)	93	12	
Nonsmokers	178(63%)	160	18	
Metastasis				0
Yes	214(75.6%)	189	25	
No	69(24.4%)	64	5	
Histopathology type				0.1599
ADC	262(93%)	237	25	
SQ	21(7%)	16	5	
Stage				0.1375
I	27(9.5%)	23	4	
II	41(14.4%)	40	1	
III	43(15.2%)	36	7	
IV	172(60.7%)	154	18	

Abbreviations: ADC, adenocarcinoma; SQ, squamous carcinoma; *TERT, telomerase reverse transcriptase*.

human *TERT* (*hTERT*) expression level, activate telomerase, maintain telomere length, and promote tumor growth.[12]

Studies have found that *TERT* co-mutation with other genes mutations can increase the degree of malignancy of tumors, consistent with an anticipated poor prognosis.[13] Co-occurring genomic alterations have emerged as core molecular and clinical heterogeneity determinants in oncogene-driven lung cancer subtypes.[14] In vitro studies have suggested that EGF upregulates the expression of RFPL3 and hTERT proteins in NSCLC cells through the MEK pathway, which promotes cell proliferation and inhibits apoptosis.[15] *KRAS* gene mutations can increase telomerase activity and TERT expression through RAS/MEK signaling, thus increasing NSCLC aggressiveness.[16] However, the prognostic significance of *TERT* co-occurrence with *EGFR/KRAS* in NSCLC is unknown and warrants further study.

Our study investigated the presence of TERT mutations in NSCLC tissues and delineated correlations with other clinical parameters. The clinical importance of *TERT* co-mutations with *EGFR* or *KRAS* in patients with NSCLC was also examined.

Materials and Methods

Patients and samples source

From September 2017 to May 2020, 283 samples from patients with NSCLC were collected from the First Affiliated Hospital of Yangtze University (Jingzhou, China). The First Affiliated Hospital of Yangtze University Ethics Committee approved the study (IRB: KY202204), and all the participants provided written informed consent. Clinicopathologic features and prognostic information were collected. Clinical characteristics are shown in Table 1. The deadline for follow-up was November 1, 2021.

Next-generation sequencing

Next-generation sequencing detected the hot spot region of 65 genes, including the whole exon region of 10 genes and 14 fusion genes, which could detect single nucleotide polymorphism

Table 2. *TERT* gene mutation type and composition ratio.

MUTATION SITE	COUNT	PERCENT (%)
P112fs-Exon2	6	20
P380S-Exon2	3	10
E113Rfs*15-exon2	5	16.7
G822S-Exon8	2	6.7
C896*-Exon11	2	6.7
P702L-Exon5	1	3.3
V554I-Exon3	1	3.3
N1057K-Exon15	1	3.3
R248Q-Exon2	1	3.3
R470L-Exon2	1	3.3
R610L-Exon4	1	3.3
V51G-Exon1	1	3.3
R889L-Exon11	1	3.3
V1035I-Exon14	1	3.3
P187Rfs*164-exon2	1	3.3
G109W-exon2	1	3.3
G963V-Exon12	1	3.3

Abbreviations: *TERT*, telomerase reverse transcriptase.

(SNP), insertion or deletion (InDels), fusions, and copy number variations in tissue samples:

> Genomic DNA from formalin-fixed paraffin-embedded samples was extracted using the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany). Nucleic acid fragmentation and terminal repair results in a specific fragment size.

Library construction was performed using the SureSelect XT Low Input kit (Agilent Technologies, Santa Clara, USA), according to the manufacturer's protocol. The captured DNA fragments were amplified with 12 to 13 cycles of PCR using 1 × KAPA HiFi Hot Start Ready Mix. In addition, Agilent Bioanalyzer (Agilent Technologies, Santa Clara, USA) quantified the library fragment size. NGS libraries were sequenced on Illumina NextSeq CN500 platform (Illumina Inc., San Diego, CA, USA).[17]

Raw image data obtained by high-throughput sequencing were transformed into raw sequences by base calling. Then, clean data were obtained by filtering adapter, low-quality reads, and reads with length < 36 bp and aligned to the reference genome (UCSC HG19) using Burrows Wheeler Aligner (BWA) to obtain BAM initial alignment result. The Genome Analysis Toolkit (GATK) containing Picard was used for local realignment, base quality recalibration, etc. Thus, the result in the BAM format and quality statistics, such as mapped reads, mean mapping quality, and mean coverage were obtained.

Finally, the HaplotypeCaller module of GATK was used to detect SNP/InDels.

Statistical analyses

The chi-square test (sample size ≥ 40, theoretical frequency < 5, Fisher's exact test) was used to analyze the association between mutations and clinical and biological characteristics. Overall survival (OS) was defined as the time from diagnosis to death or the last follow-up date for living patients. The Kaplan-Meier method was used to draw the survival curve, and the difference in survival rate was tested and analyzed using the log-rank test for statistical significance. The Cox proportional risk regression model was constructed using patients' pathological characteristics and gene mutations for multi-factor analysis. In univariate Cox regression analysis, variable screening criterion was set as $A = 0.1$, and variables with $P < 0.1$ were included in multivariate Cox regression analysis. Multivariate Cox regression analysis ($P < 0.05$) was set as an independent prognostic factor. SPSS 25.0 software (IBM Corp., Chicago, IL, USA) was used for data analysis.

Results

Patient characteristics

The sample included 283 patients with NSCLC (average age, 62 years), the majority (n = 164;58%) of whom were male and nonsmokers (n = 178, 63%). Among 283 patients, 262 (93%) were diagnosed with adenocarcinoma and 21 (7%) were diagnosed with squamous cell carcinoma. Regarding tumor staging, the numbers of patients with cancer stages I to IV was 27 (9.5%), 41 (14.4%), 43 (15.2%), and 172 (60.7%), respectively (Table 1).

TERT mutation screening in patients with NSCLC

Using NGS, we found the *TERT* mutation rate of 11% (30/283), the KRAS mutation rate of 11% (27/283), and the *EGFR* mutation rate of 55% (156/283). We identified the *TERT* gene mutation point using the sequencing data of these patients (Table 2).

The clinicopathologic characteristics of patients with *TERT* promoter mutations are presented in Table 1. *TERT* mutation carrier status was associated with sex ($P < 0.0001$), age ($P < 0.0001$), smoking history ($P < 0.0001$), and metastasis ($P < 0.0001$).

TERT and EGFR or KRAS co-mutation genomic alterations in NSCLC

We found that 17 of the patients with *TERT* mutations had *EGFR* mutations, and 3 had *KRAS* mutations (Table 3). *TERT* and *EGFR* co-mutations status was significantly correlated with sex ($P = .0105$), histopathology type ($P = .009$), and metastasis ($P = .006$). *TERT* and *KRAS* co-mutations were associated with metastasis ($P < .0001$).

Table 3. Clinical baseline table of co-mutations of *TERT* and *EGFR* or *KRAS*.

CHARACTERISTICS	*TERT* MUTATION (30)	*TERT* AND *EGFR* MUTATION (17)	*TERT* MUTATION + *EGFR* WILD (13)	*P*	*TERT* AND *KRAS* MUTATION (3)	*TERT* MUTATION + *KRAS* WILD (27)	*P* VALUE
Sex				0.0105			1
Male	17	6	11		2	15	
Female	13	11	2		1	12	
Age				0.7048			0.2793
<60	11	7	4		0	11	
≥60	19	10	9		3	16	
Histopathology type				0.009			1
ADC	25	17	8		3	22	
SQ	5	0	5		0	5	
Smoking history				0.2641			0.0542
Smokers	12	5	7		3	9	
Nonsmokers	18	12	6		0	18	
Stage				0.1345			0.7632
I	4	3	1		0	4	
II	1	0	1		0	1	
III	7	6	1		0	7	
IV	18	8	10		3	15	
Metastasis				0.006			<0.0001
Yes	25	11	14		3	22	
No	5	2	3		0	5	

Abbreviations: ADC, adenocarcinoma; *EGFR, Epidermal growth factor receptor; KRAS, Kirsten rat sarcoma virus*; SQ, squamous carcinoma; *TERT, Telomerase Reverse Transcriptase*.

Table 4. The association between *TERT* mutation carrier status and overall survival.

VARIATE	AVERAGE OS		MEDIAN OS	
	X̄ ± *SE*	95% CI	X̄ ± *SE*	95% CI
TERT wild-type	37.182 ± 2.771	31.751~42.612	30.000 ± 3.366	23.402~36.598
TERT mutation	22.792 ± 4.806	13.372~32.212	14.000 ± 4.702	4.784~23.216

Abbreviations: CI, confidence interval; OS, overall survival; *TERT, telomerase reverse transcriptase; SE*, standard error.

Clinical outcomes

The follow-up period ended on November 1, 2021. From a total of 283 patients, 161 had died at study end-point. The median survival times of the *TERT*-mutant vs TERT wild-type group was 14 vs 30 months (Table 4). There was a significant difference in OS between *TERT* mutation carriers with vs without co-mutations (P=0.0061, Figure 1A).

The median OS was 21 months in the TERT and EGFR co-mutated griou; 10 months in the TERT mutated and EGFR wild-type group; 33 months in the TERT wild-type and EGFR mutated group; and 27 months in both the TERT and EGFR wild-type group (Table 5). There was significant difference in OS among the four groups, suggestting that different groups had different effects on prognosis (P = 0.0056, Figure 1B).

The median OS was 10 months in the co-mutated TERT and KRAS group, 15 months in the wild-type TERT and mutant KRAS group, 15 months in the wild-type TERT and KRAS mutation group, and 30 months in the wild-type TERT and KRAS group (Table 6). A significant difference was found in the OS among the four groups (P = 0.012, Figure 1C). The patients with TERT and KRAS co-mutations have the worst survival.
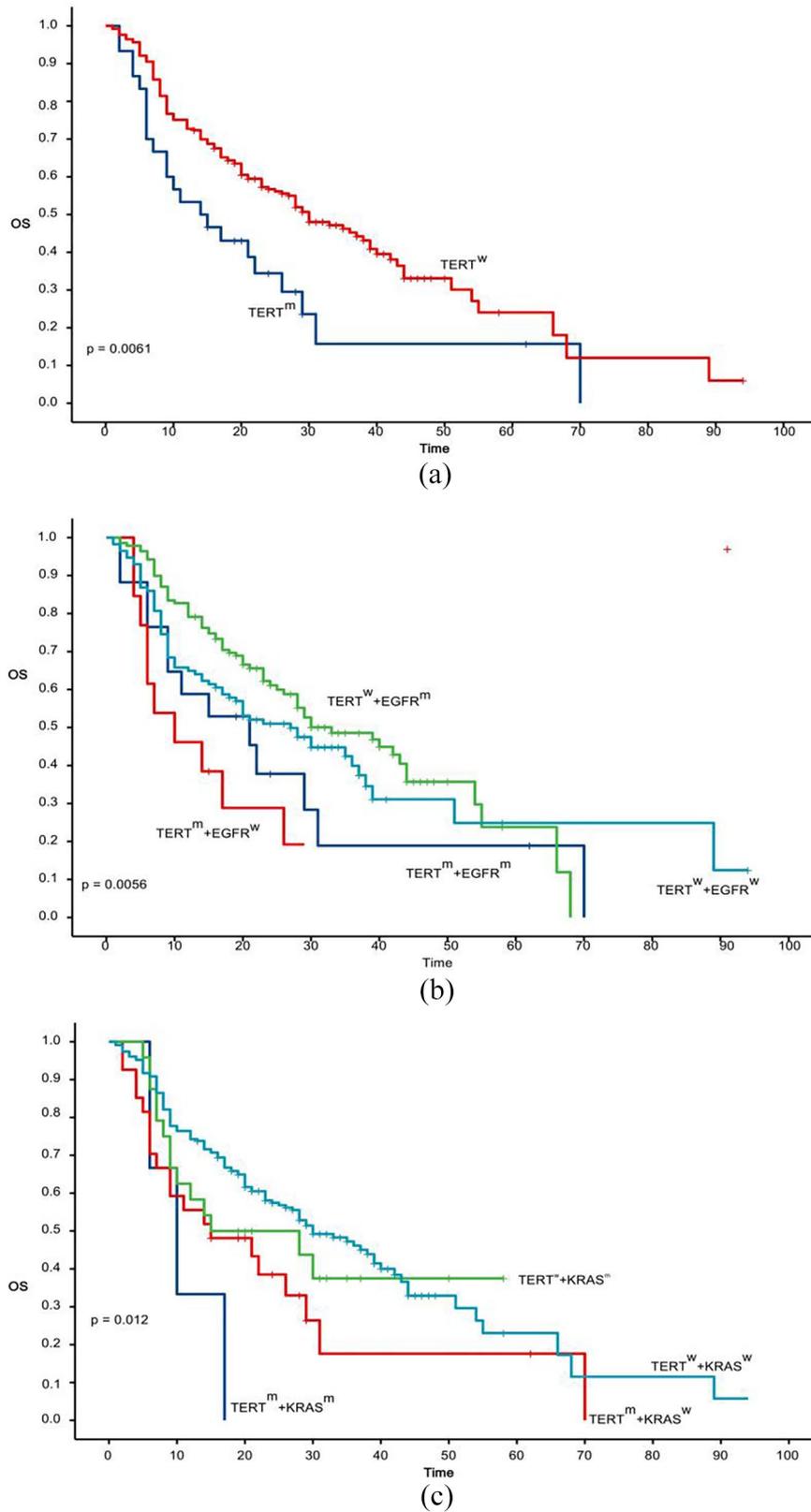
Figure 1. Overall survival for 283 patients with non–small cell lung cancer. (A) Kaplan-Meier (K-M) curve of *TERT* mutant and wild-type OS shows their association (*P* = 0.0061). (B) K-M curves of OS under different mutation states of *TERT* and *EGFR*. There were significant differences in OS (*P* = 0.0056) among the four groups in NSCLC. (C) K-M curves of OS under different mutation states of *TERT* and *KRAS* shows significant differences in OS (*P* = 0.0012) among the four groups in NSCLC.

EGFR indicates *epidermal growth factor receptor; EGFR* m, *EGFR* mutant; *EGFR* w, *EGFR* wild-type; K-M, Kaplan Meier; *KRAS, Kirsten rat sarcoma virus; KRAS* m, *KRAS* mutant; *KRAS* w, *KRAS* wild-type; NSCLC, non–small cell lung cancer; OS, overall survival; *TERT, telomerase reverse transcriptase; TERT* m, *TERT* mutant; *TERT* w, *TERT* wild-type.

Table 5. Overall survival of *TERT* and *EGFR* in different alteration status.

GROUP	AVERAGE OS		MEDIAN OS	
	X ± SE	95%CI	X ± SE	95%CI
1	25.689 ± 6.478	12.993~38.386	21.000 ± 6.554	8.153~33.847
2	14.019 ± 2.728	8.672~19.367	10.000 ± 4.793	0.605~19.395
3	36.433 ± 2.369	31.790~ 41.075	33.000 ± 5.800	21.632~44.368
4	37.624 ± 4.356	29.087~46.161	27.000 ± 4.321	18.530~35.470

Abbreviations: 1, *TERT* and *EGFR* co-mutation; 2, *TERT* mutation and *EGFR* wild-type; 3, *TERT* wild-type and *EGFR* mutation; 4, *TERT* wild-type and *EGFR* wild-type; CI, confidence interval; EGFR, *epidermal growth factor receptor*; OS, overall survival; *SE*, standard error; *TERT, telomerase reverse transcriptase*.

Table 6. Overall survival of *TERT* and *KRAS* in different alteration statuses.

GROUP	AVERAGE OS		MEDIAN OS	
	X ± SE	95% CI	X ± SE	95% CI
1	11.000 ± 3.215	4.699~17.301	10.000 ± 3.266	3.599~16.401
2	24.175 ± 5.301	13.786~34.564	15.000 ± 7.868	0.000~30.420
3	29.875 ± 4.826	20.416~39.334	15.000 ± 11.198	0.000~36.947
4	37.257 ± 2.826	31.719~42.796	30.000 ± 3.439	23.260~36.740

Abbreviations: 1, *TERT* and *KRAS* co-mutation; 2, *TERT* mutation and *KRAS* wild-type; 3, *TERT* wild-type and *KRAS* mutation; 4, *TERT* wild-type and *KRAS* wild-type; CI, confidence interval; KRAS, *Kirsten rat sarcoma virus*; OS, overall survival; *SE*, standard error; *TERT, telomerase reverse transcriptase*.

Table 7. Univariate and multivariate Cox regression analysis in NSCLC patients.

FACTORS	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	HAZARD RATIO (95% CI)	P VALUE	HR (95% CI)	*P* VALUE
Sex	0.793 (0.578-1.087)	0.15		
Age	1.021 (1.003-1.039)	0.022	1.022 (1.004-1.039)	0.014
Histopathology type	1.447 (0.835-2.507)	0.187		
Smoking history	0.815 (0.592-1.121)	0.208		
Stage	3.301 (2.494-4.370)	0.000	3.341 (2.337-4.775)	0.000
Metastasis	5.997 (3.246-11.079)	0.000	1.273 (0.588-2.775)	0.540
TERT mutation	1.834 (1.174-2.863)	0.008	2.731 (1.689-4.418)	0.000
EGFR mutation	0.767 (0.561-1.048)	0.095	0.639 (0.463-.0881)	0.06
KRAS mutation	1.340 (0.810-2.218)	0.255		
TERT + *EGFR* co-mutations	1.439 (0.810-2.555)	0.214		
TERT + *KRAS* co-mutations	3.465 (1.098-10.9377)	0.034	0.594 (0.173-2.048)	0.410

Abbreviations: CI, confidence interval; EGFR, *Epidermal growth factor receptor*; HR, hazard ratio; KRAS, *Kirsten rat sarcoma virus*; NSCLC, non–small cell lung cancer; TERT, *Telomerase Reverse Transcriptase*.

Overall survival predictors

The univariate and multivariate Cox regression analyses showed that *TERT* mutation carrier status was an independent prognostic marker in patients with NSCLC (Table 7). The results showed that age, metastasis, cancer stage, *TERT* mutation, and *TERT* and *KRAS* co-mutations were associated with worse prognosis. Meanwhile, age, stage, and *TERT* mutation exhibited potential as independent risk factors for OS.

Discussion

NSCLC is a common and highly heterogeneous malignancy, accounting for approximately 85% of all lung cancer cases, with a very low 5-year survival rate.[18] However, targeted therapy has improved 5-year survival.[19] NGS can effectively identify the status of various important lung cancer-related gene changes.[20]

TERT mutations are considered a low mutation type in NSCLC.[21] In a cohort study of 103 patients with NSCLC, using DNA gene sequencing, Yuan et al[22] suggested that the mutation rate of the *TERT* region was approximately 5.8%. Whereas Jung et al[23] reported showed that the *TERT* promoter mutation rate was 2.2% (4/188). However, our study showed that the *TERT* mutation rate in patients with NSCLC was 11%. Although the mutation rate of *TERT* was slightly higher than that reported in previous studies, the mutation rate was still low.

Our study also showed a correlation between *TERT* gene mutation carrier status, smoking history, age, sex, and distant metastasis. This result is consistent with those of a previous meta-analysis which confirmed that *TERT* gene mutations were associated with age, sex, and distant metastasis.[23]

The median survival time of the *TERT*-mutated group was 14 months, and that of the TERT wild-type group was 30 months. Similarly, there was a significant difference in OS between *TERT* mutation carriers with vs without co-mutations (*P* = 0.0061). Our results are consistent with those of the study performed by Jung et al.[23]

In an analysis of the clinicopathologic and genetic prognostic markers, we found that age, stage, and *TERT* mutation carrier status were independent risk factors for poor OS in patients with NSCLC. Among them, there were 2.731 times as many *TERT* mutation cases as there were *TERT* wild-type cases (95% CI, 1.689-4.418, <0.001). The median survival time of the *TERT*-mutant group was 14 months, and that of the *TERT* wild-type group was 30 months.

In the context of cancer, *TERT* mutations can co-occur with other risk-associated mutations in genes, such as *BRAF*, *EGFR*, *KRAS*, and *IDH* in cancer.[24-26] *TERT* mutations alone or in combination with other genetic mutations can be used to identify different tumor types and predict prognosis and outcome. In thyroid cancer, especially papillary thyroid cancer, *TERT* and *BRAF* co-mutation suggest a poor prognosis.[27] In high-grade gliomas, *TERT* and *IDH* co-mutation was associated with worse outcomes than *IDH* only.[25] *KRAS* and *EGFR* mutations are common in NSCLC. In our study, an analysis of the clinical correlates of *TERT* and *EGFR* co-mutation showed that they were related to the patient's sex, histopathology type, and metastasis. Whereas *TERT* and *KRAS* co-mutations were found to be correlated with metastasis alone.

We further analyzed the effect of *TERT* and *EGFR* co-mutations on the prognosis of patients with NSCLC. The results showed that the *TERT*-mutated and *EGFR* wild-type groups had the shortest OS, followed by the co-mutation group. In NSCLC, *KRAS* mutations increase TERT expression and telomerase activity through RAS/MEK signaling, hence contributing to the expression of more aggressive phenotypes of NSCLC.[16] Our study showed that median OS was significantly different among the *TERT* and *KRAS* co-mutation group; *TERT* mutation and *KRAS* wild-type group; *TERT* wild-type and *KRAS* mutation group; and *TERT* and *KRAS* wild-type group (*P* = 0.012). These results indicated that different groups had different prognosis. Moreover, the co-mutation of *TERT* and *KRAS* resulted in worse survival outcomes.

Our study has several limitations. First, the database had limitations in terms of investigating other potential sources of bias. *TERT, EGFR*, and *KRAS* mutation rates were all slightly higher in our study than in others. *KRAS* mutations are a major cause of NSCLC, accounting for over 30% of cases in Western countries, but less than 10% in Asia.[28] The *KRAS* mutation rate in patients with NSCLC was 11% in our study. The frequency of *EGFR* mutations in NSCLC in the People's Republic of China ranges from 28.2% to 43%.[29] In our study, the *EGFR* mutation rate was 55%. Difference in *TERT* mutation incidence between our study and others may be related to the choice of sample size and region. Due to financial constraints, not all patients attending the clinic underwent genetic testing. Second, our patients were mainly recruited from rural areas and had limited access to treatment, which affected their OS. We found worse survival in the *TERT* and *EGFR* co-mutation group than in the *EGFR* mutation and *TERT* wild-type group. We hypothesized that this might be related to the use of tyrosine kinase inhibitor preparations in most *EGFR* mutation-positive patients; however, because not all patients were treated in the same way, we did not include the treatment of patients in the study, which may have influenced the results. Therefore, prospective studies are needed to confirm this finding.

Conclusions

In conclusion, we have illustrated the incidence and clinicopathologic correlates of *TERT* mutations in patients with NSCLC. We also demonstrated that age, stage, and TERT mutations were independent risk factors for poor prognosis in patients with NSCLC. This result provides a reference for the clinical evaluation of prognosis in patients with NSCLC.

Author Contributions

XCP, YHY, and JC contributed to this paper with the design and reviewed the submitted version of the manuscript. YHY, LY, WZ, and LDY worked on the acquisition, analysis and interpretation of the data. XCP, JC, YHY, NL, and LY worked on the statistical analysis. LY, MW, and NL, YZQ contributed to tables and figures. LY, JC, YHY, and XCP wrote the manuscript. XCP and JC acquired funding. All authors contributed to the article and approved the submitted version.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Yangtze University and complied with Good Clinical Practices (IRB: KY202204). The patients/participants provided their written informed consent to participate in this study.

Availability of Data and Materials

The original data sheet of our experiment results can be found in the supporting information.

ORCID iD

Liu Yang https://orcid.org/0000-0001-7530-9588

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