계명의대학술지
Keimyung Medical Journal

Original Article

pISSN 2092-8335 · eISSN 2733-5380
Keimyung Med J 2020 39(1):23-27
https://doi.org/10.46308/kmj.2020.00108

Received: January 16, 2020
Revised: May 11, 2020
Accepted: May 14, 2020

Corresponding Author:
Jae-Ho Lee, M.D.
Department of Anatomy, Keimyung University School of Medicine, 1095 Dalgubeol-daero, Dalseo-gu, Daegu 42601, Korea
Tel:+82-53-258-7512
E-mail: anato82@dsmc.or.kr

Absence of Clinical Value of TZAP Mutation and Expression in Non-small Cell Lung Cancers

Deok Heon Lee[1], Soo-Jung Jung[2], Jae-Ho Lee[2]

[1]Department of Thoracic and Cardiovascular Surgery, Kyungpook National University School of Medicine, Daegu, Korea
[2]Department of Anatomy, Keimyung University School of Medicine, Daegu, Korea

The zinc finger protein ZBTB48 is a telomere-associated factor and renamed it as telomeric zinc finger-associated protein (TZAP). It binds preferentially to long telomeres competing with TRF1 and TRF2. However, its mutation in cancers has not been studied. In the present study, we analyzed TZAP mutation in 134 non-small cell lung cancers (NSCLCs). And its big data analysis was performed using COSMIC and TCGA data analysis. TZAP mutation was not found in 134 NSCLCs. And big data also showed that TZAP mutation was extremely low (0.59%, 15/2548). TCGA survival analysis showed no prognostic value of TZAP expression in lung adenocarcinoma (p = 0.185) and squamous cell carcinoma (p = 0.817). When stratified patients sorting as 25:25 (quarter), it has a significance (p = 0.003). This result suggested that genetic change of TZAP did not appear to be a possible molecular marker in lung cancer.

Keywords: Non-small cell lung cancers, Telomere, TZAP, ZBTB48

Introduction

Telomeres, composed of 6-bp TTAGGG repeat sequences, are nucleoprotein complexes capping each end of eukaryotic chromosomes [1,2]. In normal human somatic cells, telomeres have an average length of 5 to 15 kilobases and are shortened by approximately 30 to 200 base pairs at every cell division. Telomere shortening is counteracted by the reverse transcriptase telomerase in stem cells and most types of cancer, while the remaining cancers maintain telomeres with alternative lengthening of telomeres (ALT) mechanism [3-5]. A telomere trimming mechanism is induced in the presence of overly long telomeres, which are cut back to normal length by rapid telomere shortening [6-8]. Though the specific regulation of this process has not been identified, recent studies have discovered a special protein that is necessary for regulating telomere [9]. They identified the zinc finger protein ZBTB48, as a telomere-associated factor, and renamed it telomeric zinc finger-associated protein (TZAP). It binds preferentially to long telomeres competing with telomeric repeat factor 1 and 2 (TRF1 and TRF2). In addition, the study showed that overexpression of TZAP caused progressive telomere shortening [9]. TZAP localizes to chromosome 1p36, a region that is frequently rearranged or deleted in various cancers [10-12]. This genetic change of TZAP may be associated with cancer pathogenesis, however, a genetic analysis of TZAP has not been performed in any specific type of cancer.

Lung cancer is the leading cause of cancer-related deaths worldwide, with only 16.8% of lung cancer patients alive 5 years after diagnosis [13]. Many

causative factors for lung cancer have been identified, including active smoking, secondhand smoke, occupational agents, radiation, and environmental pollutants [14-15]. These factors may influence the telomere regulation in human inducing various disease such as especially in nonsmall cell lung cancer (NSCLC). These previous studies suggested that the regulation of telomere length had the potential to serve as prognostic marker for patients with NSCLC, and may therefore aid clinicians in making informed therapeutic decision [16,17]. Therefore, we analyzed TZAP mutation and expression in patients with NSCLC for the first time. To increase the statistical significance, we confirmed big cohorts, The Cancer Genome Atlas (TCGA) and Catalogue of Somatic Mutation In Cancer (COSMIC) data.

Materials and Methods

Patients and tissue samples

A total of 134 cases were identified from pathology reports of patients who underwent conventional surgery for NSCLC at the Kyungpook National University Hospital (KNUH). All materials derived from the National Biobank of Korea, KNUH, were obtained under institutional review board-approved protocols (Approval No. KNUMCBIO_14-1010). None of the patients received chemotherapy or radiotherapy prior to surgery. We collected basic clinical data including age, gender, disease stage, and smoking status. The pathologic staging of lung cancer was based on the 7th AJCC staging system.

DNA samples of the tumor tissue and adjacent normal mucosa were obtained using an extraction kit according to the manufacturer's instructions (Absolute™ DNA extraction Kit, BioSewoom, Korea). The quantity and quality of DNA were measured using NanoDrop 1000 (Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA).

TZAP mutation analysis

TZAP exons were amplified from isolated DNA using the polymerase chain reaction (PCR). PCR was performed using AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The sequences of primers for all exons are presented in Table 1. Thermocycling was conducted under the following conditions: 40 cycles of 94°C for 30 sec, 55-57°C for 30 sec, and 72°C for 60 sec. The PCR products were separated on a 1.5% agarose gel using electrophoresis and stained with ethidium bromide for 20 min to confirm the size of the bands. Direct DNA sequencing of the TZAP was subsequently performed using an ABI 3730 DNA sequencer (Bionics Inc., Seoul, South Korea).

The Cancer Genome Atlas (TCGA) data analysis

To investigate the clinical significance of TZAP, we used the TCGA and COSMIC database. TZAP mRNA expression data were downloaded from TCGA's data portal (https://tcga-data.nci.nih.gov/tcga/) on October, 2019 [18]. And TZAP mutation data were checked form COSMIC (https://cancer.sanger.ac.uk/cosmic) on October, 2019 [19]. Its clinicopathological and prognostic values were analyzed.

Statistical analysis

Statistical analysis was performed by SPSS version 23.0 (IBM SPSS, Armonk, NY, USA). Survival curves, constructed using the univariate Kaplan-Meier estimators, were compared using the log-rank test. Overall survival (OS) was defined as the time between diagnosis and mortality. A p value of <0.05 denoted significance for all statistical analyses performed in this study.

Results

The sequences of TZAP regions were successfully analyzed in the 134 NSCLCs tissue samples. We found no TZAP muta-

Table 1. Primer sequences used in this study

Name	Primer sequences
TZAP exon 1	F: CCAGACCTCAACAGCACAGA
	R: CACAGCCCACGAACCTAGTG
TZAP exon 2	F: ATCCCATTGGCCGTTCTCT
	R: CCGGCACAGTGAGAGGAT
TZAP exon 3	F: TAGAGGCCAACTTCCCGTTT
	R: CCTGGGCACAGTACCTCATT
TZAP exon 4	F: CCTGCTGATTCATTTGGTGA
	R: GGAATGGCAGACAGGAAAAG
TZAP exon 5-1	F: GGAGGTGAGGAAGTTGACCA
	R: CCCTTCTAAGGGGAACAAGTG
TZAP exon 5-2	F: GCTTGTCCCTGCACCTTAAC
	R: GGAGAGGGCAACACATAACC
TZAP exon 5-3	F: AGTCTGTCTGGGCCTGAGAA
	R: CCCTCCCTGTCACTTACTGC
TZAP exon 6	F: CCCTTCCCTGCTCTCACC
	R: AAGAGAGAACGGGCGACAC
TZAP exon 7	F: GTCACTTCCCTTGGTGATGG
	R: GAGGGGACCAGTGGTTTACA
TZAP exon 8	F: CTGGGTGGCACTGGAGAG
	R: CACGGGAACAGACTGTCAGG

tions in all NSCLCs. In COSMIC data, this mutation was found in 0.58% (15/2548) of NSCLC. It was summarized in Table 2. Most mutations were missense mutation and it did not have any clinical significance.

We then assessed the survival analysis to clarify the prognostic significance of the TZAP expression in patients with NSCLC using TCGA data. In 488 squamous cell carcinomas, TZAP expression did not show any prognostic value for the patients with NSCLCs (p = 0.817, Fig. 1A). In 246 adenocarcinomas, TZAP also did not have any prognostic significance (p = 0.185, Fig. 1B). However, when the patients were divided by quarter, poorer survival results were found in lower expression group (p = 0.003, Fig. 1C).

Discussion

In this study, we demonstrated TZAP mutation and expression for the first time in cancer, specifically NSCLC. TZAP mutations have not yet been studied and rare mutations were only reported in the International Cancer Genome Consortium (ICGC) data [19]. In our hospital samples, we found no TZAP mutation in lung cancer and non-cancerous tissue, respectively. COSMIC data also reported an extremely lower frequency of TZAP mutation in lung cancers. Thus, this mutation may be not specific for lung cancer, although this needs to be further confirmed.

ZBTB48 is a Kruppel-like C2H2 zinc finger protein consisting of 11 tandem zinc finger domains located C-terminal to the BTB/POZ domain [9]. Recent study established that ZBTB48, renamed by Li et al. [9] as TZAP for telomeric zinc finger-associated protein, tend to bind overly long telomeres in mouse stem cells and cancer cells, irrespective of whether telomerase or ALT is operational. TZAP appears to compete with TRF1 and TRF2 for binding to telomeres, and its association could be contingent on the exhaustion of free shelterin, possibly as telomeres replicate and elongate [8,9]. Therefore, its mutation or expression may be associated with various diseases, especially cancers. Based on this hypothesis, we searched ZBTB48 (TZAP) expression level in TCGA data and analyzed its prognostic value in lung cancers.

According to the TCGA data, TZAP expression, unfortunately, was not associated with lung cancer survival. However, it showed a possibility for prognostic value in some classification. Our study has demonstrated, for the first time, a better prognosis in patients with NSCLC with higher TZAP expression. However, TZAP did not have any clinical and prognostic values totally. Considering the important role of telomere regulation in lung cancer [12,16,17], precise mechanism of genetic change of TZAP should be studied further.

In conclusion, we studied the TZAP mutation and TZAP expression in patients with NSCLC. TZAP did not have great significance for clinical and prognostic markers in NSCLC. The results of the present study warrant future large-scale studies to elucidate the underlying molecular mechanisms of TZAP and to determine the potential clinical utility.

Table 2. COSMIC data of TZAP (ZBTB48) mutation in lung cancers

Sample Name	ID	Substitution			Histology
		Amino acid	Nucleotide	Type	
HOP-62	1998445	p.S89	c.266C>G	Nonsense	AD
IGC-02-1163	2662279	p.R548L	c.1643G>T	Missense	AD
IGC-04-1086	2662299	p.H407Y	c.1219C>T	Missense	AD
LC_S19	1863730	p.K307N	c.921A>T	Missense	AD
LUAD_E00522	1765259	p.I678M	c.2034C>G	Missense	AD
824_T	2194936	p.R485C	c.1453C>T	Missense	SCC
H157	2776238	p.L157V	c.469C>G	Missense	SCC
J76_T	2195003	-	c.1379+12C>G	Intron variant	SCC
TCGA-22-5472-01	1780945	p.V562	c.1686G>T	coding silent	SCC
TCGA-34-5929-01	1781318	p.S258	c.773C>G	Nonsense	SCC
2014_Lung_sq_06_T	2744877	-	c.1379+12C>G	Intron variant	Unknown
2014_Lung_sq_41_T	2744912	p.R485C	c.1453C>T	Missense	Unknown
2014_Lung_sq_88_T	2744855	-	c.933-238C>T	Intron variant	Unknown
TCGA-91-6830-01	1914092	p.S187F	c.560C>T	Missense	AD

AD, adenocarcinoma; SCC, squamous cell carcinoma.

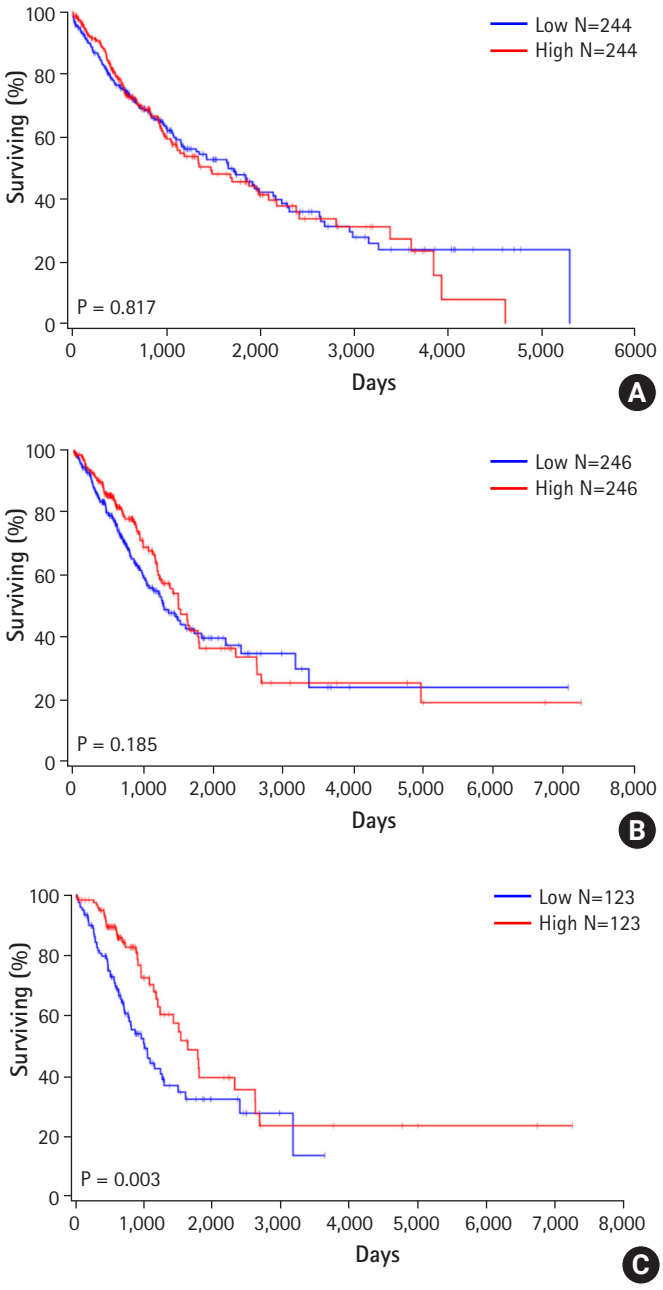

Fig. 1. TCGA data of TZAP expression (ZBTB48) in NSCLC. (A) Overall survival of TZAP expression in squamous cell carcinoma (B) Overall survival of TZAP expression in adenocarcinoma sorted by half (50:50) (C) Overall survival of TZAP expression in adenocarcinoma sorted by quarter (25:25).

Conflict of interest

All authors declare no conflicts-of-interest related to this article.

References

1. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990;345:458-60.
2. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nat Med. 2006;12:1133-8.
3. Xin B, Liu D, Songyang Z. The telosome/shelterin complex and its functions. Genome Biol. 2008 9. DOI: 10.1186/gb-2008-9-9-232.
4. Shay JW, Wright WE. Role of telomeres and telomerase in cancer. Semin Cancer Biol. 2011;21:349-53.
5. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016;8. DOI: 10.1186/s13073-016-0324-x.
6. Pickett HA, Cesare AJ, Johnston RL, Neumann AA, Reddel RR. Control of telomere length by a trimming mechanism that involves generation of t-circles. EMBO J. 2009;28:799-809.
7. Pickett HA, Reddel RR. The role of telomere trimming in normal telomere length dynamics. Cell Cycle 2012;11:1309-15.
8. Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. Science. 2012;336:593-7.
9. Li JSZ, Miralles Fusté J, Simavorian T, Bartocci C, Tsai J, Karlseder J, et al. TZAP: a telomere-associated protein involved in telomere length control. Science. 2017;355:638-41.
10. Maris JM, Jensen SJ, Sulman EP, Beltinger CP, Gates K, Allen C, et al. Cloning, chromosomal localization, physical mapping, and genomic characterization of HKR3. Genomics. 1996;35:289-98.
11. White PS, Maris JM, Sulman EP, Jensen SJ, Kyemba SM, Beltinger CP, et al. Molecular analysis of the region of distal 1p commonly deleted in neuroblastoma. Eur J Cancer. 1997;33:1957-61.
12. Maris JM, Jensen J, Sulman EP, Beltinger CP, Allen C, Biegel JA, et al. Human Krüppel-related 3 (HKR3): a candidate for the 1p36 neuroblastoma tumour suppressor gene? Eur J Cancer. 1997;33:1991-6.
13. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64:9-29.
14. Alberg AJ, Brock MV, Ford JG, Samet JM, Spivack SD. Epidemiology of lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013;143:e1S–e29S
15. Wu CY, Hu HY, Pu CY, Huang N, Shen HC, Li CP, et al. Pulmonary tuberculosis increases the risk of lung cancer: a population-based cohort study. Cancer. 2011;117:618-24.
16. Lee DH, Heo YR, Park WJ, Lee JH. A TERT-CLPTM1 locus polymorphism (rs401681) is associated with EGFR mutation in non-small cell lung cancer. Pathol Res Pract. 2017;213:1340-3.

17. Jung SJ, Kim DS, Park WJ, Lee H, Choi IJ, Park JY, et al. Mutation of the TERT promoter leads to poor prognosis of patients with non-small cell lung cancer. Oncol Lett. 2017;14:1609-14.
18. National Cancer Institute. The Cancer Genome Atlas Program. [cited 2020 Mar 16]. Available from: https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga
19. Sanger institute. COSMIC, Catalogue Of Somatic Mutations In Cancer. [cited 2020 Mar 16]. Available from: https://cancer.sanger.ac.uk/cosmic/mutation/overview?id=101113777