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Original Article

EXO1 is a Potential Prognostic Biomarker in Lung Cancer

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Abstract : Lung cancer is one of the most common malignancies worldwide and remains the leading cause of cancer-related deaths. Exonuclease 1 (EXO1) is involved in several critical biological processes, including mismatch repair, double-strand break repair, and nucleotide excision repair. However, its precise role and association with lung cancer (LC) have not been fully elucidated. In this study, we investigated the clinicopathological and prognostic significance of EXO1 mRNA expression in 102 patients with LC. No statistically significant associations were observed between EXO1 expression and clinicopathological variables. However, survival analysis revealed that low EXO1 expression was significantly associated with poorer overall survival. Notably, among patients with squamous cell carcinoma (SCC), those with low EXO1 expression had significantly shorter overall survival compared to those with high expression levels. Our findings suggest that EXO1 may serve as a potential prognostic biomarker, particularly in SCC.

Keywords : EXO1, TCGA, Lung cancer, Biomarker

BACKGROUND

Lung cancer is one of the most common diseases worldwide and the leading cause of cancer-related deaths. According to Global Cancer Statistics 2022, lung cancer was estimated to account for approximately 2.5 million new cases and over 1.8 million deaths worldwide, representing nearly one in eight (12.4%) cancer diagnoses and one in five (18.7%) cancer-related deaths globally. In terms of both incidence and mortality, the disease ranks first in men and second in women. The five-year survival rate for lung cancer is typically below 20% in most countries, primarily attributed to factors such as late diagnosis, frequent recurrence, and metastasis [1]. Several risk factors for lung cancer have been identified, including smoking, occupational exposures, genetic component, radiation, and environmental pollutants. Among these factors, smoking history is the predominant risk factor for lung cancer development [2]. In recent years, a diversity of innovative treatment methods, including surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy, have been progressively utilized in clinics [3-5]. However, despite these advances, lung cancer continues to be the leading cause of cancer-related mortality and has one of the lowest five-year survival rates across all cancer types. This underscores the urgent need for reliable prognostic biomarkers to facilitate early diagnosis and guide more effective treatment strategies.

Exonuclease 1 (EXO1), located on chromosome 1q42-43, comprises a non-coding exon and 13 coding exons that encode a protein of 846 amino acids [6,7]. EXO1 is a member of the Rad2/XPG family of exonucleases and includes an N-terminal nuclease domain and an interaction domain [8,9]. The nuclease region is composed of two subdomains: the N-terminal ('N') domain, which mediates DNA binding,

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and the internal ('I') domain, which contains multiple cysteine and glutamate residues essential for Mg^{2+} binding [10]. The EXO1 gene product exhibits 5′→3′ exonuclease activity, 5′-endonuclease activity and RNase H activity. It also interacts directly with key mismatch repair proteins, including MLH1 and MSH2, forming ternary complexes such as EXO1-MLH1-PMS2 and EXO1-MSH2-MSH6 [11-16]. Functionally, EXO1 plays critical roles in multiple biological processes, including DNA mismatch repair (MMR), double-strand break repair (DSBR), nucleotide excision repair (NER), immunoglobulin maturation, and telomere maintenance [17-22]. Additionally, EXO1 has been implicated in the regulation of telomerase activity and cell survival. Knockdown of EXO1 has been shown to reduce telomerase activity and cell viability, suggesting its significant involvement in cancer cell growth and survival [23]. Deletion of the EXO1 gene leads to genomic instability, impaired DNA damage repair, and meiotic defects [24-27].

Numerous studies have demonstrated that aberrantly high expression of EXO1 is closely associated with the development, progression, metastasis, and prognosis of various malignancies. For instance, in hepatocellular carcinoma, high EXO1 expression correlates with several clinical parameters and contributes to poor prognosis [28]. Bioinformatics analyses have also revealed a positive correlation between EXO1 and FOXP3 expression, supporting its role in tumor progression [29]. Similarly, elevated EXO1 levels are linked to reduced overall survival in breast cancer [30] and are associated with various clinicopathological features, further validating its potential as a biomarker for disease progression [31]. High EXO1 expression has also been associated with poor prognosis in prostate cancer [32] and astrocytoma [33].

In this study, we investigated the clinical and prognostic significance of EXO1 mRNA expression in lung cancer tissues. Our findings provide important insights into the role of EXO1 in lung cancer pathogenesis and may contribute to the development of novel prognostic and therapeutic strategies.

METHODS

1. Patients and samples

A total of 102 patients diagnosed with lung cancer were included in this study. Samples were obtained from the Keimyung Human Bioresource Bank, Korea. Data were obtained from patients who underwent surgery at the Dongsan Medical Center (Daegu, Korea) between April 2010 and January 2016. Our study complied with the Declaration of Helsinki. All patients were informed of the study purpose and informed consent was obtained from each participant before the research was conducted. Clinicopathological data of each patient were re-evaluated during a review of their medical records. TNM staging of lung cancer was used according to the 8th AJCC staging system. This study was approved by the Institutional Review Board of Keimyung University Dongsan Medical Center (No. 2020-07-027).

2. RNA isolation and mRNA expression analysis

RNA was extracted from samples using TRIzol reagent (Molecular Research Center Inc., Cincinnati, OH, USA) according to the manufacturer's protocol. RNA quantity and quality were measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, Denmark). The expression levels of EXO1 were measured using RT-qPCR (Bio-Rad, Hercules, CA, USA). Each experiment was performed in duplicate. The PCR amplification cycles were as follows: 95°C for 10 min, 134 followed by 40 cycles of 95°C for 60 s and 72°C for 30 s. The sequences of the EXO1 primers were forward, 5′-TCGGATCTCCTAGC TTTTGGCTG-3′ and reverse, 5′-AGCTGTCTGCACATTC CTAGCC-3′.

3. Statistical analysis

All statistical analyses were performed using The Statistical Package for the Social Sciences (SPSS), version 24.0, for Windows (IBM, Armonk, NY, USA). Chi-square was used to analyze the relationships between variables. The mean gene expression level was used as a cutoff to stratify patients into high- and low-expression groups for survival analysis. The Kaplan-Meier method was used for survival analysis, and the log rank test was performed to evaluate statistically significant differences between the two groups. Statistical significance was defined as a two-tailed P value <0.05.

RESULTS

The median value of EXO1 expression was 2.68 ± 2.78 in a cohort of 102 patients and they were stratified into two groups based on the median expression level of EXO1. The associ-ations between EXO1 expression and various clini-

Table 1. Clinical significance of EXO1 expression in lung cancers

	EXO1		
	Low	High	*P*-value
Total	74 (72.5)	28 (27.5)	
Age			
<65	27 (36.5)	14 (50)	0.214
≥65	47 (63.5)	14 (50)	
Gender			
Female	16 (21.6)	6 (21.4)	0.983
Male	58 (78.4)	22 (78.6)	
Smoking			
No	16 (21.6)	6 (21.4)	0.983
Yes	58 (78.4)	22 (78.6)	
Location			
LLL	16 (21.6)	5 (17.85)	
LUL	15 (20.3)	5 (17.85)	
RLL	14 (18.9)	7 (25)	0.603
RML	5 (6.8)	0 (0)	
RUL	24 (32.4)	11 (39.3)	
Histology			
AD	35 (47.3)	11 (39.3)	
SCC	31 (41.9)	11 (39.3)	0.370
Others	8 (10.8)	6 (21.4)	
Differentiation			
Poorly	14 (25.5)	6 (33.3)	0.515
Well/Moderate	41 (74.5)	12 (66.7)	
T stage			
T1	25 (33.8)	4 (14.3)	
T2	31 (41.9)	19 (67.9)	0.117
T3	11 (14.9)	3 (10.7)	
T4	7 (9.4)	2 (7.1)	
N stage			
N0	51 (68.9)	25 (89.3)	
N1	14 (18.9)	2 (7.1)	0.108
N2	9 (12.2)	1 (3.6)	
M stage			
M0	74 (100)	28 (100)	–
M1	0 (0)	0 (0)	
Pathological stage			
I	33 (44.6)	18 (64.3)	
II	27 (36.5)	7 (25)	0.202
III	14 (18.9)	3 (10.7)	
EGFR mutation			
(+)	13 (68.4)	1 (16.7)	0.260
(−)	6 (31.6)	5 (83.3)	

copathological parameters—including age, gender, smoking history, tumor location, histological type, tumor differentiation, TNM stage, pathological stage, and EGFR mutation status—are summarized in Table 1. There were no statistically significant differences in EXO1 expression with respect to age ($P=0.214$), gender ($P=0.983$), smoking history ($P=0.983$), or tumor location ($P=0.603$). Although EXO1 expression appeared to be slightly associated with T stage, the difference did not reach statistical significance ($P=0.117$). Similarly, no significant correlations were observed between EXO1 expression and other parameters, including histology ($P=0.370$), tumor differentiation ($P=0.515$), pathological stage ($P=0.202$), or EGFR mutation status ($P=0.260$).

Table 2. Correlation analysis of EXO1 expression according to age, tumor size, and overall survival

		EXO1	Age	Tumor size	Survival days
EXO1	r	1			
	P-value				
Age	r	−.130	1		
	P-value	.192			
Tumor size	r	.042	.043	1	
	P-value	.677	.595		
Survival days	r	.029	−.181	−.162	1
	P-value	.773	.026	.046	

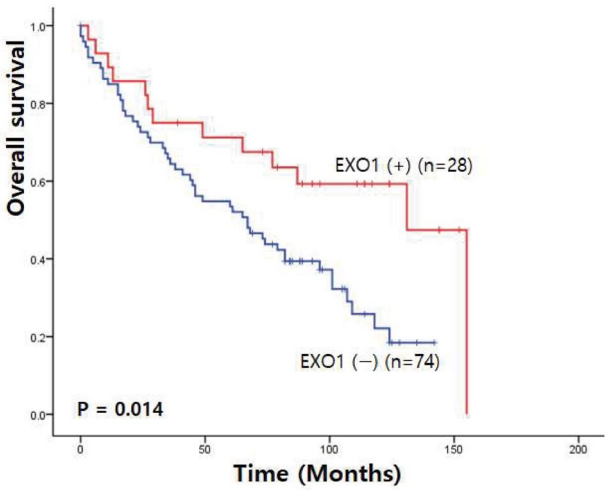

Fig. 1. Survival analysis in lung cancer according to EXO1 expression.

Quantitative correlation analysis further confirmed that EXO1 expression was not significantly associated with age ($P=0.192$), tumor size ($P=0.677$), or survival duration

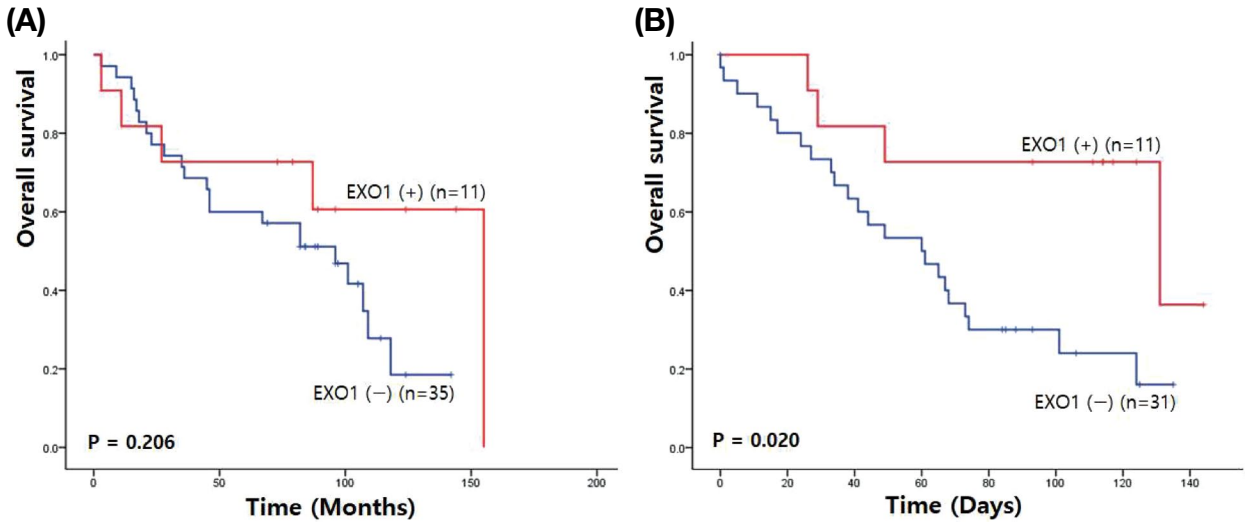

Fig. 2. Survival analysis in lung cancer according to histological classification. (A) adenocarcinoma and (B) squamous cell carcinoma.

($P=0.773$). However, age and tumor size were both negatively correlated with overall survival ($P=0.026$ and $P=0.046$, respectively), as shown in Table 2.

To evaluate the prognostic value of EXO1 expression, Kaplan-Meier survival analysis was performed. Patients with low EXO1 expression exhibited significantly poorer overall survival compared to those with high expression levels ($P=0.014$; Fig. 1). When stratified by histological subtype, survival analysis revealed that low EXO1 expression was significantly associated with reduced overall survival in patients with squamous cell carcinoma (SCC) ($P=0.020$; Fig. 2). In contrast, no significant prognostic value was observed for EXO1 expression in adenocarcinoma ($P=0.206$) or other subtypes ($P=0.586$).

DISCUSSION

In this study, we analyzed the expression of EXO1 to explore its clinical relevance in lung cancer by using patient-derived tissue samples. Our findings provide valuable insights into the potential role of EXO1 in lung cancer progression and prognosis. EXO1 has been reported to play a pivotal role in various biological processes, including DNA mismatch repair (MMR), double-stranded break repair (DSBR), and telomere maintenance [34,35]. Loss of EXO1 function has been associated with genomic instability and tumor progression, factors that are linked to poor survival outcomes in lung cancer [36,37]. Despite this, the precise role of EXO1 in lung cancer pathogenesis remains largely unclear.

To address this gap, we conducted a comprehensive analysis of the clinicopathological characteristics and prognostic implications of EXO1 expression in lung cancer patients. While previous studies have established EXO1 as a key component of DNA repair mechanisms and a potential biomarker in various malignancies [15,21,22], this study aims to specifically elucidate its prognostic significance in lung cancer.

We assessed EXO1 expression levels through RT-qPCR analysis of clinical samples. Our clinical data revealed that EXO1 expression did not significantly differ across various clinicopathological parameters, including age, gender, smoking history, tumor location, TNM stage, pathological stage, and EGFR mutation status. Although a minor association was observed between EXO1 expression and T stage, it did not reach statistical significance. Notably, a previous study in lung adenocarcinoma (AD) demonstrated significant correlations between EXO1 expression and T, N, and M stages [38]. Another study reported that overexpression of EXO1 was linked to advanced T stage and reduced overall survival (OS) in AD patients [39]. These findings suggest that overexpressed EXO1, a key regulator of DNA repair and replication stress responses, may promote tumor proliferation and invasiveness, contributing to disease progression.

Interestingly, survival analysis showed that low EXO1 expression was significantly associated with poorer overall survival, where EXO1 overexpression typically correlated

with worse prognosis [39,40]. In patients with squamous cell carcinoma (SCC), low EXO1 expression was significantly linked to poorer survival outcomes. However, in AD and other subtypes, EXO1 expression did not show a statistically significant correlation with survival. These results highlight the biological heterogeneity of lung cancer, which encompasses diverse histological subtypes and molecular profiles [41]. Given that SCC and AD originate from distinct cell types and exhibit different biomarker landscapes, the functional role of EXO1 may vary accordingly. Our findings suggest that EXO1 may be particularly relevant in the pathogenesis of SCC.

EXO1 may influence lung tumorigenesis through its involvement in DNA replication and other critical cellular processes. Previous studies have shown that EXO1 may regulate tumor immune escape mechanisms. However, EXO1 has also demonstrated a positive correlation with multiple immune activators, promoting immune responses [42,43]. These findings, along with our results, suggest that the immunomodulatory effect of EXO1 is highly cancer-specific, indicating its potential dual role. This discrepancy highlights the need for a deeper understanding of the mechanisms by which EXO1 functions in cancer, providing an important biological basis for future cancer treatment strategies.

However, this study has several limitations. The findings have been only partially validated using clinical tissue samples, and the relatively small sample size may have introduced bias. Furthermore, the detailed molecular mechanisms by which EXO1 contributes to lung cancer development remain to be elucidated. Future studies involving larger patient cohorts and functional assays are warranted to further clarify the role of EXO1 in lung cancer.

Given the potential dual role of EXO1 in tumor immunity—both promoting immune responses and contributing to immune evasion—a more comprehensive investigation is required to better understand its cancer-specific immunomodulatory functions. Such insights will provide a stronger biological basis for the development of EXO1-targeted therapeutic strategies.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74:229-63.
2. Lo YL, Hsiao CF, Chang GC, Tsai YH, Huang MS, Su WC, et al. Risk factors for primary lung cancer among never smokers by gender in a matched case-control study. Cancer Causes Control. 2013;24:567-76.
3. Camidge DR, Doebele RC, Kerr KM. Comparing and contrasting predictive biomarkers for immunotherapy and targeted therapy of NSCLC. Nat Rev Clin Oncol. 2019;16:341-55.
4. Lee JW, Lee DY, Kim DH. Knowledge and attitude about alternative treatment in cancer patient. Keimyung Med J. 2023;42:38-43.
5. Hwang SH, Kim J. Comprehensive analysis of chromobox 1 expression, DNA methylation and non-coding RNA interactions in lung adenocarcinoma. Keimyung Med J. 2025;44:74-86.
6. Imtiaz H, Afroz S, Hossain MA, Bellah SF, Rahman MM, Kadir MS, et al. Genetic polymorphisms in CDH1 and Exo1 genes elevate the prostate cancer risk in Bangladeshi population. Tumour Biol. 2019;41:1010428319830837.
7. Zhang M, Zhao D, Yan C, Zhang L, Liang C. Associations between nine polymorphisms in EXO1 and cancer susceptibility: a systematic review and meta-analysis of 39 case-control studies. Sci Rep. 2016;6:29270.
8. Keijzers G, Bohr VA, Rasmussen LJ. Human exonuclease 1 (EXO1) activity characterization and its function on flap structures. Biosci Rep. 2015;35:pii:e00206.
9. Wilson DM, Coleman MA, Adamson AW, Christensen M, Lamerdin JE, Carney JP. Hex1: a new human Rad2 nuclease family member with homology to yeast exonuclease 1. Nucleic Acids Res. 1998;26:3762-8.
10. Orans J, McSweeney EA, Iyer RR, Hast MA, Hellinga HW, Modrich P, et al. Structures of human exonuclease 1 DNA complexes suggest a unified mechanism for nuclease family. Cell. 2011;145:212-23.
11. Lee BI, Nguyen LH, Barsky D, Fernandes M, Wilson DM. Molecular interactions of human Exo1 with DNA. Nucleic Acids Res. 2002;30:942-9.
12. Schmutte C, Sadoff MM, Shim KS, Acharya S, Fishel R. The interaction of DNA mismatch repair proteins with human exonuclease I. J Biol Chem. 2001;276:33011-8.
13. Schmutte C, Marinescu RC, Sadoff MM, Guerrette S, Overhauser J, Fishel R. Human exonuclease I interacts with the mismatch repair protein hMSH2. Cancer Res. 1998;58:4537-42.
14. Liberti SE, Andersen SD, Wang J, May A, Miron S, Perderiset M, et al. Bi-directional routing of DNA mismatch repair protein human exonuclease 1 to replication foci and DNA double strand breaks. DNA Repair. 2011;10:73-86.
15. Chen ZY, Zheng SR, Zhong JH, Zhuang XD, Zhou JY. Association between three exonuclease 1 polymorphisms and cancer risks: a meta-analysis. Onco Targets Ther. 2016;9:899-910.

16. Jäger AC, Rasmussen M, Bisgaard HC, Singh KK, Nielsen FC, Rasmussen LJ. HNPCC mutations in the human DNA mismatch repair gene hMLH1 influence assembly of hMut Lalpha and hMLH1-hEXO1 complexes. Oncogene. 2001;20: 3590-5.
17. Keijzers G, Liu D, Rasmussen LJ. Exonuclease 1 and its versatile roles in DNA repair. Crit Rev Biochem Mol Biol. 2016;51:440-51.
18. Keijzers G, Bakula D, Petr MA, Madsen NGK, Teklu A, Mkrtchyan G, et al. Human exonuclease 1 (EXO1) regulatory functions in DNA replication with putative roles in cancer. Int J Mol Sci. 2018;20:74.
19. Xue Y, Marvin ME, Ivanova IG, Lydall D, Louis EJ, Maringele L. Rif1 and Exo1 regulate the genomic instability following telomere losses. Aging Cell. 2016;15:553-62.
20. Goellner EM, Putnam CD, Kolodner RD. Exonuclease 1-dependent and independent mismatch repair. DNA Repair. 2015;32:24-32.
21. Bolderson E, Tomimatsu N, Richard DJ, Boucher D, Kumar R, Pandita TK, et al. Phosphorylation of Exo1 modulates homologous recombination repair of DNA double-strand breaks. Nucleic Acids Res. 2010;38:1821-31.
22. Cotta-Ramusino C, Fachinetti D, Lucca C, Doksani Y, Lopes M, Sogo J, et al. Exo1 processes stalled replication forks and counteracts fork reversal in checkpoint-defective cells. Mol Cell. 2005;17:153-9.
23. Luo Z, Wang W, Li F, Songyang Z, Feng X, Xin C, et al. Pan-cancer analysis identifies telomerase-associated signatures and cancer subtypes. Mol Cancer. 2019;18:106.
24. Wang S, Lee K, Gray S, Zhang Y, Tang C, Morrish RB, et al. Role of EXO1 nuclease activity in genome maintenance, the immune response and tumor suppression in Exo1D173A mice. Nucleic Acids Res. 2022;50:8093-106.
25. Stroik S, Kurtz K, Lin K, Karachenets S, Myers CL, Bielinsky AK, et al. EXO1 resection at G-quadruplex structures facilitates resolution and replication. Nucleic Acids Res. 2020;48: 4960-75.
26. Wei K, Clark AB, Wong E, Kane MF, Mazur DJ, Parris T, et al. Inactivation of exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes Dev. 2003;17:603-14.
27. Schaetzlein S, Chahwan R, Avdievich E, Roa S, Wei K, Eoff RL, et al. Mammalian Exo1 encodes both structural and catalytic functions that play distinct roles in essential biological processes. Proc Natl Acad Sci U S A. 2013;110:E2470-9.
28. Dai Y, Tang Z, Yang Z, Zhang L, Deng Q, Zhang X, et al. EXO1 overexpression is associated with poor prognosis of hepatocellular carcinoma patients. Cell Cycle. 2018;17:2386-97.
29. Yang G, Dong K, Zhang Z, Zhang E, Liang B, Chen X, et al. EXO1 plays a carcinogenic role in hepatocellular carcinoma and is related to the regulation of FOXP3. J Cancer. 2020;11: 4917-32.
30. Qi L, Zhou B, Chen J, Hu W, Bai R, Ye C, et al. Significant prognostic values of differentially expressed-aberrantly methylated hub genes in breast cancer. J Cancer. 2019;10:6618-34.
31. Liu J, Zhang J. Elevated EXO1 expression is associated with breast carcinogenesis and poor prognosis. Ann Transl Med. 2021;9:135.
32. Luo F, Wang YZ, Lin D, Li J, Yang K. Exonuclease 1 expression is associated with clinical progression, metastasis, and survival prognosis of prostate cancer. J Cell Biochem. 2019; 120:11383-9.
33. de Sousa JF, Torrieri R, Serafim RB, Di Cristofaro LFM, Escanfella FD, Ribeiro R, et al. Expression signatures of DNA repair genes correlate with survival prognosis of astrocytoma patients. Tumour Biol. 2017;39:1010428317694552.
34. Szankasi P, Smith GR. A role for exonuclease I from S. pombe in mutation avoidance and mismatch correction. Science. 1995;267:1166-9.
35. Maringele L, Lydall D. EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Δ mutants. Genes Dev. 2002;16:1919-33.
36. Tomimatsu N, Mukherjee B, Harris JL, Boffo FL, Hardebeck MC, Potts PR, et al. DNA-damage-induced degradation of EXO1 exonuclease limits DNA end resection to ensure accurate DNA repair. J Biol Chem. 2017;292:10779-90.
37. Jeon HS, Choi YY, Choi JE, Lee WK, Lee E, Yoo SS, et al. Telomere length of tumor tissues and survival in patients with early stage non-small cell lung cancer. Mol Carcinog. 2014; 53:272-9.
38. Sun B, Zhang J, Wang N, Zhang Z, Wu Y, Xie M, et al. The bioinformatics analysis and experimental validation of the carcinogenic role of EXO1 in lung adenocarcinoma. Front Oncol. 2024;14:1492725.
39. Zhou CS, Feng MT, Chen X, Gao Y, Chen L, Li LD, et al. Exonuclease 1 (EXO1) is a potential prognostic biomarker and correlates with immune infiltrates in lung adenocarcinoma. Onco Targets Ther. 2021;14:1033-48.
40. Wang S, Cai W, Li J, An W, Zheng H, Liao M. Bioinformatics analysis and experimental study of exonuclease 1 gene in lung adenocarcinoma. Biochem Genet. 2022;60:1934-45.
41. Zheng M. Classification and pathology of lung cancer. Surg Oncol Clin N Am. 2016;25:447-68.
42. Lin X, Kang K, Chen P, Zeng Z, Li G, Xiong W, et al. Regulatory mechanisms of PD-1/PD-L1 in cancers. Mol Cancer. 2024;23:108.
43. Ai L, Chen J, Yan H, He Q, Luo P, Xu Z, et al. Research status and outlook of PD-1/PD-L1 inhibitors for cancer therapy. Drug Des Devel Ther. 2020;14:3625-49.