International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.874.882

Research Article
EGFR and K-Ras Gene Mutations in the Diagnosis of Non-Small Cell Lung Cancer and their Pathological Correlation

Zhenhua Wu, Waresijiang Yibulayin, Dan He, Keming Xu, Xiayimaierdan Yibulayin, Lei Ma and Xiaohong Sun

Department of Thoracic Surgery, Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi 830011, Xinjiang, China

Abstract

Background and Objective: The incidence of Non-Small Cell Lung Cancer (NSCLC) has been steadily increasing in recent years and its subtle clinical manifestations in the early stages lead to poor patient prognosis. Gene mutations are associated with the occurrence and progression of NSCLC. This study aimed to investigate the value of EGFR and K-ras gene mutations in the diagnosis of NSCLC and analyze their correlation with pathological characteristics of patients. **Materials and Methods:** A total of 180 patients, 90 were taken as control while other 90 patients diagnosed with NSCLC from August 2020 to January 2023 were selected as the study group. The detection rates of EGFR and K-ras gene mutations were compared between the two groups. The main phenotypes of EGFR and K-ras gene mutations in the study group were analyzed and the correlation between the gene mutations and the pathological characteristics of the patients (degree of differentiation, presence or absence of lymph node metastases, histological typing, patient gender, etc.) were analyzed. **Results:** The gene mutation rates of EGFR and K-ras were significantly higher in the study group than in the control group. In 90 patients with NSCLC, the mutation rate of EGFR gene mutations was 48.89%, while the mutation rate of K-ras gene mutations was 12.22%. The EGFR gene mutation rate was significantly higher in adenocarcinoma than in squamous cell carcinoma ($p<0.05$) and the degree of differentiation was significantly correlated with the EGFR gene mutation rate ($p<0.05$). **Conclusion:** The gene mutation rates of EGFR and K-ras in NSCLC patients were significantly higher than those of benign lung lesions. There was a certain correlation between the EGFR gene mutation rate and the pathological characteristics of the patients, which should be verified in further studies.

Key words: EGFR gene mutation, K-ras gene mutation, non-small cell lung cancer, pathological correlation, diagnostic value

Citation: Wu, Z., W. Yibulayin, D. He, K. Xu, X. Yibulayin, L. Ma and X. Sun, 2024. EGFR and K-ras gene mutations in the diagnosis of non-small cell lung cancer and their pathological correlation. Int. J. Pharmacol., 20: 874-882.

Corresponding Author: Xiaohong Sun, Department of Thoracic Surgery, Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi 830011, Xinjiang, China Tel: +86–0991-7819332

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the Updated Global Cancer Burden Data 2020 by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), the number of deaths caused by cancer has reached 9.96 million worldwide, with a total of 1.8 million patients suffering from lung cancer, which is far more than any other types of cancer, ranking first in the number of cancer deaths[1]. The number of cancer deaths in China reached 3 million in 2020, among which the number of lung cancer deaths has reached 710,000, accounting for about 23.8% of cancer deaths[2]. All of the above data show that lung cancer has become the tumor that poses the greatest threat to human health in the world[3]. Lung cancer can be differentiated into small-cell lung cancer (15-20%) and Non-Small Cell Lung Cancer (NSCLC) (80-85%) according to pathological types, of which the main types of NSCLC include squamous lung cancer, adenocarcinoma of the lung, large cell lung cancer, etc.[4]. According to the practice, the main reason for the high mortality rate of NSCLC is that the early symptoms of the disease are not obvious and about 75% of patients are already in the middle to late stage of lung cancer when they are detected; even if they have received active surgical, radiotherapy and chemotherapy treatments[5], the 5-year survival rate of patients with NSCLC is only 10-15%. Therefore, early diagnosis and intervention are important means to improve the prognosis of the patients.

In recent years, targeted drugs for epidermal growth factor receptor (EGFR) have been widely used in the treatment of NSCLC, which has greatly prolonged the survival time and improved the quality of life of patients[6]. A further research has indicated that targeted drugs are more effective in patients with EGFR exon mutations, which makes it possible to predict patient outcomes by detecting gene mutations[7]. The gene K-ras is a gene downstream of the EGFR signal transduction pathway that acts as a switch during EGFR signaling and the activation of K-ras allows cells to bypass the EGFR signaling pathway and directly activate the downstream kinase system, thus inducing tumor[8]. All of the above studies have laid a certain theoretical foundation for the diagnosis of NSCLC by detecting EGFR and K-ras genes. This study innovatively demonstrated the value of EGFR and K-ras gene mutations in the diagnosis of NSCLC, as well as in the evaluation of pathological characteristics, by means of control analysis.

MATERIALS AND METHODS

Materials: Lung tissues of 236 patients who underwent lung puncture or surgical resection in Affiliated Tumor Hospital of Xinjiang Medical University from August 2020 to January 2023 were retrospectively screened from the in-hospital information system. A total of 236 lung tissues were screened, including 98 NSCLC tissues and 138 benign lesion tissues and secondary screening was performed with reference to the following inclusion criteria.

Inclusion criteria: (1) The tissues diagnosed with NSCLC underwent pathological examination with clear pathological characteristic information (histological typing, degree of differentiation, presence or absence of lymph node metastases, etc.), (2) None of the patients diagnosed with NSCLC were treated with chemoradiotherapy before sampling and (3) All tissues were tested for EGFR gene and K-ras gene.

Finally, 90 NSCLC tissues meeting the inclusion criteria were determined and set as the study group and 90 benign lesion tissues were randomly selected as the control group to ensure the best fit.

Methods

DNA extraction: Paraffin-embedded tumor tissues were taken and 5-10 slices with a thickness of 5-6 μm were cut. After dewaxing with xylene, ethanol was added to elute xylene. After drying, lysate and protease were added and incubated at 55°C for 3 hrs and protease was inactivated at 100°C for 10 min. The saturated sodium chloride solution (120 μL) was added and the supernatant was taken after shaking and centrifuging. Two times the volume of anhydrous ethanol was added and placed at -20°C for precipitation for 2 hrs and then centrifuged at 4°C for 20 min. After removing the lower precipitate, 80% cold ethanol was added for centrifugation at 4°C for 20 min and the precipitate was vacuum dried.

PCR amplification: The primer sequences for exons 18-21 of EGFR gene, as well as the primer sequences for codons 12-13 and codon 61 of K-ras gene were shown in Table 1. The 25 μL PCR reactions were performed and the PCR reaction procedure of EGFR was as follows: Pre-denaturation at 95°C for 5 min, 35 cycles, denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, primer extension at 72°C for 60 sec and finally extension at 72°C for 10 min. The reaction procedure of codons 12-13 of K-ras gene was as follows: Pre-denaturation at 95°C for 10 min, 35 cycles, denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec, primer extension at 72°C for 40 sec and finally extension at 72°C for 10 min. The reaction procedure of codon 61 of K-ras gene was as follows: Pre-denaturation at 95°C for 10 min, 35 cycles, denaturation at 94°C for 30 sec, annealing at 47°C for 30 sec, primer extension

Table 1: Primer sequences of the genes EGFR and K-ras

Genes	Primer sequences
EGFR	
Exon 18	5'-CAAATGAGCTGGCAAGTGCCGTGTC-3'
	5'-GAGTTTCCCAAACACTCAGTGAAAC-3'
Exon 19	5'-GCAATATCAGCCTTAGGTGCGGCTC-3'
	5'-CATAGAAAGTGAACATTTAGGATGTG-3'
Exon 20	5'-CCATGAGTACGTATTTTGAAACTC-3'
	5'-CATATCCCCATGGCAAACTCTTGC-3'
Exon 21	5'-CTAACGTTCGCCAGCCATAAGTCC-3'
	5'-GCTGCGAGCTCACCCAGAATGTCTGG-3'
K-ras	
Codons 12-13	5'-TCAAAGAATGGTCCTGCACC-3'
	5'-GCCTGCTGAAAATGACTGAA-3'
Codon 61	5'-CTTGGATATTCTCGACACAGCTGAT-3'
	5'-AACTATAATTACTCCTTAATGTCAGCTTA-3'

at 72°C for 40 sec and finally extension at 72°C for 10 min. The above operations were strictly in accordance with the DNA kit instructions.

Gene sequencing: After performing the PCR according to the above procedures, the products were precipitated and purified using ethanol and finally sequenced using the NovaSeq 6000 sequencer.

Statistical methods: The data were collected using EXCEL 2020 and analyzed using SPSS 22.0. The diagnostic efficacy was analyzed by plotting ROC curves and the differences in the detection of EGFR and K-ras mutations were compared using the Chi-square test. The $p<0.05$ was defined as the level of significance.

Ethical consideration: This study was conducted under the approval of the Ethics Committee of Affiliated Tumor Hospital of Xinjiang Medical University. Each procedure was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all the patients at the beginning of the treatment, allowing the use of clinical data in the further research.

RESULTS

Comparison of EGFR and K-ras gene mutation rates between the study and control groups: In the patients of the study group, the EGFR gene mutation rate was 48.89% (44/90) and the K-ras gene mutation rate was 12.22% (11/90), which were significantly higher than those of the control group (5.56% (5/90) and 1.11% (1/90), respectively) ($p<0.05$) (Table 2, Fig. 1).

Diagnostic efficacy of EGFR and K-ras mutations in NSCLC: The ROC curves of EGFR and K-ras gene mutations for the diagnosis of NSCLC were plotted respectively and the calculation showed that the AUC of EGFR gene mutations for the diagnosis of NSCLC was 0.7278 (95% CI = 0.6525-0.8031, $p<0.0001$) and the AUC of K-ras gene mutations for the diagnosis of NSCLC was 0.9333 (95% CI = 0.8911-0.9755, $p<0.0001$) (Fig. 2).

Analysis of EGFR gene mutation sites in patients with NSCLC: A total of 44 out of 90 patients with NSCLC had EGFR gene mutations, with a mutation rate of 48.89%, including 6 (13.64%) cases with exon 18 mutations, 5 (11.36%) cases with exon 19 mutations, 5 (11.36%) cases with exon 20 mutations and 6 (13.64%) cases with exon 21 mutations (Fig. 3).

Analysis of K-ras gene mutation sites in patients with NSCLC: A total of 11 out of 90 patients with NSCLC had K-ras gene mutations, with a mutation rate of 12.22%, including 6 (13.64%) cases with exon 18 mutations, 5 (11.36%) cases with exon 19 mutations, 5 (11.36%) cases with exon 20 mutations and 6 (13.64%) cases with exon 21 mutations (Fig. 4).

Correlation between EGFR gene mutations and pathological characteristics in patients with NSCLC: The EGFR gene mutation rate was significantly higher in adenocarcinoma than in squamous cell carcinoma ($p<0.05$) and the degree of differentiation was significantly correlated with the EGFR gene mutation rate ($p<0.05$). The presence or absence of lymph node metastasis did not significantly affect the EGFR gene mutation rate ($p>0.05$) (Table 3, Fig. 5).

Correlation between K-ras gene mutations and pathological characteristics in patients with NSCLC: There was no correlation between K-ras gene mutations and pathological characteristics such as gender, histological typing and degree of differentiation of the patients ($p>0.05$) (Table 4, Fig. 6).

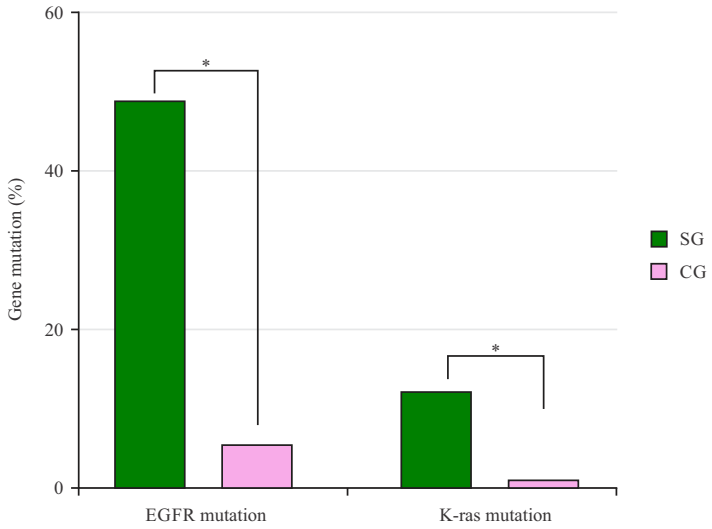

Fig. 1: Comparison of EGFR and K-ras gene mutation rates between the study and control groups
Compared with the control group and *p<0.05

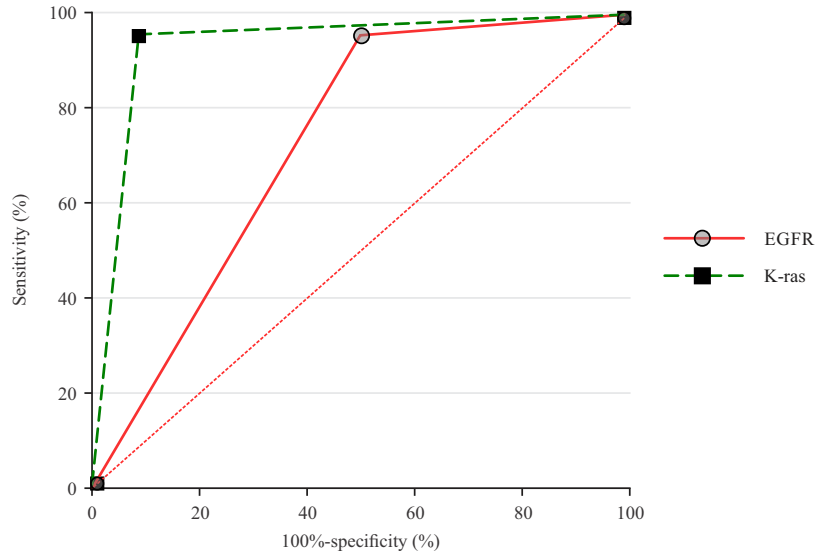

Fig. 2: Diagnostic efficacy of EGFR and K-ras mutations in NSCLC

Table 2: Comparison of EGFR and K-ras gene mutation rates between the study and control groups

Group	Case	EGFR mutation	K-ras mutation
Study group	90	44 (48.89)	11 (12.22)
Control group	90	5 (5.56)	1 (1.11)
χ^2	-	6.986	3.659
p	-	0.000	0.016

DISCUSSION

In this study, a comparative analysis was conducted and it was found that EGFR and K-ras mutation rates were significantly higher in patients with NSCLC compared with those with benign lung lesions and the clinical efficacy of EGFR and K-ras mutations in the diagnosis of EGFR was further verified through plotting ROC curves. In a study of 136 patients with histologically confirmed NSCLC, Shiri *et al.*[9] found that EGFR mutations and K-ras mutations had a better

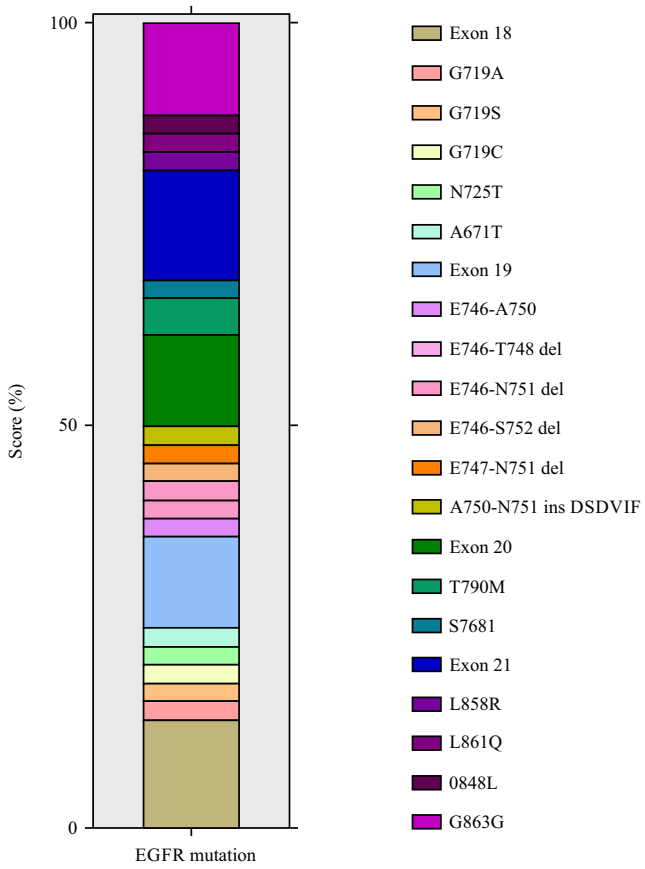

Fig. 3: Analysis of EGFR gene mutation sites in patients with NSCLC

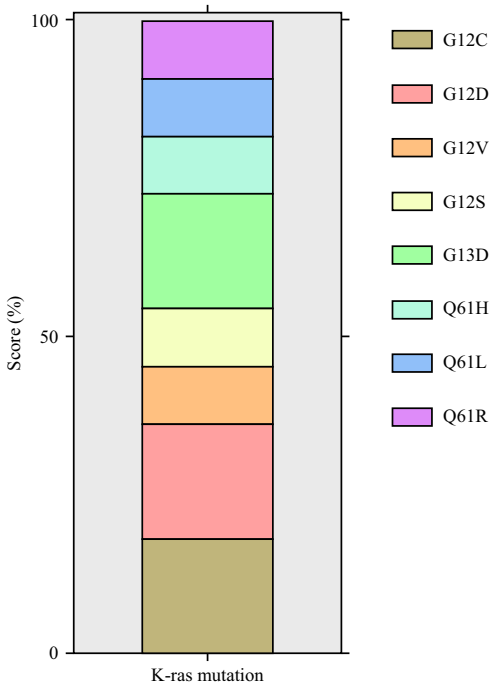

Fig. 4: Analysis of K-ras gene mutation sites in patients with NSCLC

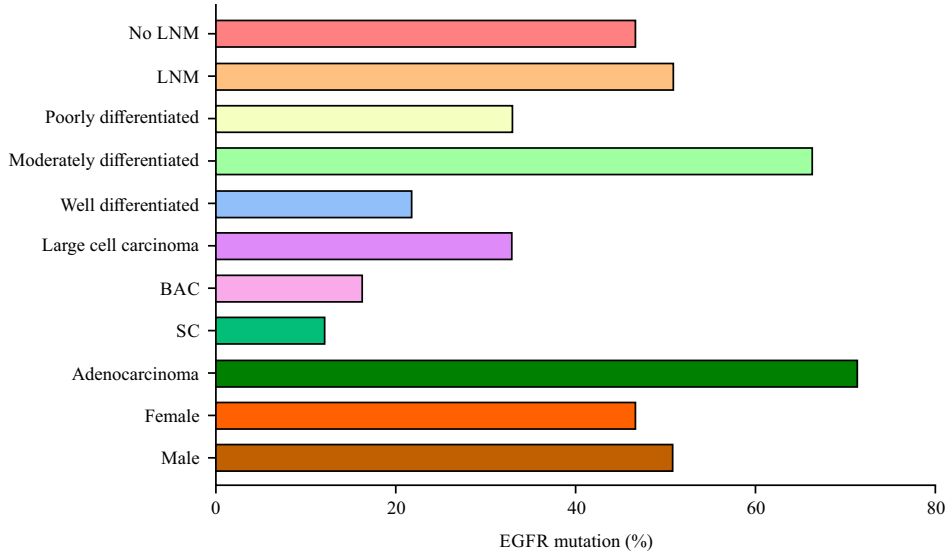

Fig. 5: Correlation between EGFR gene mutations and pathological characteristics in patients with NSCLC

Table 3: Correlation between EGFR gene mutations and pathological characteristics in patients with NSCLC

Pathological characteristics	n	Number of EGFR mutations	EGFR mutation rate (%)	χ^2	p
Gender					
Male	41	21	51.22	1.231	0.361
Female	49	23	46.94		
Histological typing					
Adenocarcinoma	53	38	71.70	5.669	0.004
Squamous cell carcinoma	16	2	12.50		
Bronchioloalveolar carcinoma	18	3	16.67		
Large cell carcinoma	3	1	33.33		
Degree of differentiation					
High	27	6	22.22	4.136	0.016
Intermediate	51	34	66.67		
Low	12	4	33.33		
Lymph node metastasis					
Yes	41	21	51.22	0.698	0.469
No	49	23	46.94		

diagnostic efficacy for NSCLC before being coordinated by the multivariate model of the random forest classifier and the diagnostic efficacy of EGFR and K-ras mutations for NSCLC was significantly improved after the ComBat coordination, with the AUC of EGFR diagnosis increasing from 0.87-0.90 to 0.92-0.94 and the AUC of K-ras diagnosis increasing from 0.85-0.90 to 0.91-0.94.

Tumor is a disease caused by genetic mutations, which are widespread in the body, but constant involvement of genetic mutations leads to abnormalities in tumor-related signaling pathways in the body, thus inducing the development of tumor[10]. Tumors are highly heterogeneous. Genotype differences between the same tumor, different tumors and different patients lead to the flexible selection of genotypes for intervention according to the actual situation of patients during the treatment process. In addition, tumors are in a constant state of evolution and changes in tumor gene spectrum have been an important reason for tumor resistance in recent years[11]. The NSCLC is the histological type of lung cancer that excludes small cell carcinoma, which accounts for approximately 80% of all lung cancers[12,13]. Compared with small cell carcinoma, non-small cell carcinoma tumor tissue divides slowly and spread later, but its clinical symptoms are not obvious, so about 75% of patients are already in the middle and late stage when they are detected, leading to a higher fatality rate, which puts forward higher expectations for the diagnosis and identification of early non-small cell carcinoma[14].

The continuous advancement of molecular biology technology in recent years has enabled genetic detection to play an important role in the diagnosis and identification of various cancers[15]. Many studies have also found that gene

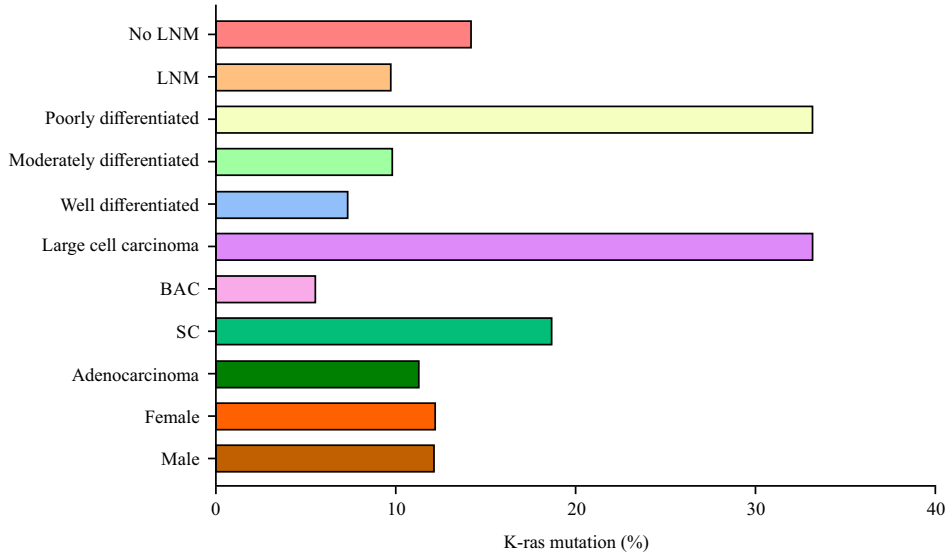

Fig. 6: Correlation between K-ras gene mutations and pathological characteristics in patients with NSCLC

Table 4: Correlation between K-ras gene mutations and pathological characteristics in patients with NSCLC

Pathological characteristics	n	Number of K-ras mutations	K-ras mutation rate (%)	χ^2	p
Gender					
Male	41	5	12.20	0.635	0.441
Female	49	6	12.24		
Histological typing					
Adenocarcinoma	53	6	11.32	0.698	0.362
Squamous cell carcinoma	16	3	18.75		
Bronchioloalveolar carcinoma	18	1	5.56		
Large cell carcinoma	3	1	33.33		
Degree of differentiation					
High	27	2	7.41	0.416	0.881
Intermediate	51	5	9.80		
Low	12	4	33.33		
Lymph node metastasis					
Yes	41	4	9.76	0.369	0.719
No	49	7	14.29		

mutations are associated with pathological characteristics of tumor patients[16], providing new ideas and references for early identification of cancers as well as the determination of treatment options. This is also the theoretical foundation and initial design intention of this study.

The EGFR is a glycoprotein receptor on the surface of the cell membrane with tyrosine kinase activity and belongs to the expression products of proto-oncogenes, which are able to bind to extracellular ligands and form dimers, which, after further phosphorylation and transphosphorylation, stimulate signaling transduction to the cell body, ultimately triggering a cascade reaction[17]. If the EGFR gene is mutated, it is involved in the processes of tumor cell proliferation, angiogenesis, tumor invasion and metastasis by affecting the signaling pathways[18]. The K-ras gene is a downstream signaling pathway of EGFR that has an inhibitory effect on the growth of tumor cells and this gene has been verified to have a sustained stimulatory effect on cell growth if mutated, ultimately inducing the occurrence of tumor[19]. These mechanisms have confirmed the correlation of EGFR and K-ras gene mutations with the development and progression of NSCLC.

The specific sites of EGFR and K-ras gene mutations in patients with NSCLC were further analyzed, with the findings similar to those of other scholars. Yang *et al.*[20] found that EGFR-tyrosine kinase inhibitors could be used in the treatment of NSCLC patients with EGFR gene mutations and it was found through molecular biology that EGFR-tyrosine kinase inhibitors exerted the therapeutic effects precisely by acting on the exon 18-21 of the EGFR gene. In a meta-analysis, Zhang *et al.*[21] pointed out that immune checkpoint inhibitors had good efficacy in the treatment of NSCLC patients with

K-ras gene mutation (HR = 0.61, 95% CI = 0.39-0.94), positive efficacy in the treatment of patients with EGFR gene mutation (HR = 0.67, 95% CI = 0.60-0.67) and were more effective than chemotherapy (HR = 0.64, 95% CI = 0.55-0.75); the meta-analysis has also indicated that there are various types of immune checkpoint inhibitors and it is recommended that patients with NSCLC be tested for genetic mutations before intervention therapy.

Finally, the correlation of EGFR and K-ras gene mutations with pathological characteristics of NSCLC patients was analyzed and the results showed that EGFR gene mutation rate in adenocarcinoma was significantly higher than that in squamous cell carcinoma ($p<0.05$), the degree of differentiation was significantly correlated with the EGFR gene mutation rate ($p<0.05$) and lymph node metastasis did not significantly affect the EGFR gene mutation rate ($p>0.05$); There was no correlation between K-ras gene mutation and pathological characteristics such as gender, histological type and degree of differentiation ($p>0.05$). These results are also similar to the research findings of other scholars[22], but there are also studies[23] that contradict the results of the present study. The authors of this study believed that influenced by the sample size and the large difference in numbers of samples of different tissue typing, the single-center, small-sample study was only able to provide reference, rather than a definitive guide, for the results of EGFR and K-ras mutations in determining the clinical treatment regimen for patients with NSCIC. However, if the sample size can be increased, it will help reduce the error of the study results and provide more reference for the determination of clinical treatment options for NSCLC patients.

CONCLUSION

The gene mutation rates of EGFR and K-ras in NSCLC patients were significantly higher than those of benign lung lesions, with EGFR gene mutation showing a higher detection rate and K-ras gene mutation showing a lower detection rate. There was a certain correlation between the EGFR gene mutation rate and the pathological characteristics of the patients, which should be verified in further studies. In the future, it is planned to conduct a multicenter study by expanding the sample size to thoroughly analyze the correlation between the mutation rates of EGFR and K-ras genes and the pathological characteristics as well as the prognosis of NSCLC patients, so as to provide additional reference data for improving the clinical outcomes of NSCLC patients.

SIGNIFICANCE STATEMENT

This study addresses the pressing need for improved diagnostic methods in NSCLC, the leading cause of cancer-related mortality worldwide. By investigating the mutation rates of EGFR and K-ras genes in NSCLC patients, diagnostic efficacy was evaluated and their association with pathological characteristics. Current findings revealed significantly higher mutation rates of EGFR and K-ras genes in NSCLC patients compared to benign lesions, with EGFR mutations exhibiting a higher detection rate. Moreover, this study provided insights into the correlation between gene mutations and pathological characteristics, laying groundwork for personalized treatment strategies. This research highlights the pivotal role of genetic testing in enhancing early detection and prognostication in NSCLC, thereby contributing crucial knowledge to the advancement of cancer care.

REFERENCES

1. Aran, V. and J. Omerovic, 2019. Current approaches in NSCLC targeting K-RAS and EGFR. Int. J. Mol. Sci., Vol. 20. 10.3390/ijms20225701.
2. Chevallier, M., P. Tsantoulis, A. Addeo and A. Friedlaender, 2020. Influence of concurrent mutations on overall survival in EGFR-mutated non-small cell lung cancer. Cancer Genomics Proteomics, 17: 597-603.
3. Chu, Q.S., 2020. Targeting non-small cell lung cancer: Driver mutation beyond epidermal growth factor mutation and anaplastic lymphoma kinase fusion. Ther. Adv. Med. Oncol., Vol. 12. 10.1177/1758835919895756.
4. Cortellini, A., B. Ricciuti, H. Borghaei, A.R. Naqash and A. D'Alessio *et al*., 2022. Differential prognostic effect of systemic inflammation in patients with non-small cell lung cancer treated with immunotherapy or chemotherapy: A *post hoc* analysis of the phase 3 OAK trial. Cancer, 128: 3067-3079.
5. da Silva-Oliveira, R.J., I.N.F. Gomes, L.S. da Silva, A. van Helvoort Lengert and A.C. Laus *et al*., 2022. Efficacy of combined use of everolimus and second-generation pan-EGRF inhibitors in *KRAS* mutant non-small cell lung cancer cell lines. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms23147774.
6. Deng, L.L., G. Gao, H.B. Deng, F. Wang, Z.H. Wang and Y. Yang, 2019. Co-occurring genetic alterations predict distant metastasis and poor efficacy of first-line EGFR-TKIs in EGFR-mutant NSCLC. J. Cancer Res. Clin. Oncol., 145: 2613-2624.
7. Huang, R.S.P., L. Harries, B. Decker, M.C. Hiemenz and K. Murugesan *et al*., 2022. Clinicopathologic and genomic landscape of non-small cell lung cancer brain metastases. Oncologist, 27: 839-848.

8. Jaromi, L., V. Csongei, M. Vesel, E.M.M. Abdelwahab and A. Soltani *et al*., 2021. KRAS and EGFR mutations differentially alter ABC drug transporter expression in cisplatin-resistant non-small cell lung cancer. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22105384.
9. Shiri, I., M. Amini, M. Nazari, G. Hajianfar and A.H. Avval *et al*., 2022. Impact of feature harmonization on radiogenomics analysis: Prediction of EGFR and KRAS mutations from non-small cell lung cancer PET/CT images. Comput. Biol. Med., Vol. 142. 10.1016/j.compbiomed.2022.105230.
10. Kosibaty, Z., O.T. Brustugun, I.J.Z. Eide, G. Tsakonas and O. Grundberg *et al*., 2022. Ras-related protein Rab-32 and thrombospondin 1 confer resistance to the EGFR tyrosine kinase inhibitor osimertinib by activating focal adhesion kinase in non-small cell lung cancer. Cancers, Vol. 14. 10.3390/cancers14143430.
11. Pan, Y., H. Han, H. Hu, H. Wang and Y. Song *et al*., 2023. *KMT2D* deficiency drives lung squamous cell carcinoma and hypersensitivity to RTK-RAS inhibition. Cancer Cell, 41: 88-105.E8.
12. Pinheiro, G., T. Pereira, C. Dias, C. Freitas and V. Hespanhol *et al*., 2020. Identifying relationships between imaging phenotypes and lung cancer-related mutation status: *EGFR and KRAS*. Sci. Rep., Vol. 10. 10.1038/s41598-020-60202-3.
13. Qu, G.P., M. Shi, D. Wang, J.H. Wu, P. Wang, M.L. Gong and Z.J. Zhang, 2021. Dual targeting of MEK and PI3K effectively controls the proliferation of human EGFR-TKI resistant non-small cell lung carcinoma cell lines with different genetic backgrounds. BMC Pulm. Med., Vol. 21. 10.1186/s12890-021-01571-x.
14. Rizzo, S., S. Raimondi, E.E.C. de Jong, W. van Elmpt and F. de Piano *et al*., 2019. Genomics of non-small cell lung cancer (NSCLC): Association between CT-based imaging features and *EGFR* and *K-RAS* mutations in 122 patients-An external validation. Eur. J. Radiol., 110: 148-155.
15. Scheffler, M., M.A. Ihle, R. Hein, S. Merkelbach-Bruse and A.H. Scheel *et al*., 2019. K-ras mutation subtypes in NSCLC and associated co-occuring mutations in other oncogenic pathways. J. Thoracic Oncol., 14: 606-616.
16. Shi, Y., Y. Lei, L. Liu, S. Zhang and W. Wang *et al*., 2021. Integration of comprehensive genomic profiling, tumor mutational burden, and PD-L1 expression to identify novel biomarkers of immunotherapy in non-small cell lung cancer. Cancer Med., 10: 2216-2231.
17. Sitthideatphaiboon, P., C. Teerapakpinyo, K. Korphaisarn, N. Leelayuwatanakul and N. Pornpatrananrak *et al*., 2022. Co-occurrence *CDK4/6* amplification serves as biomarkers of de novo EGFR TKI resistance in sensitizing *EGFR* mutation non-small cell lung cancer. Sci. Rep., Vol. 12. 10.1038/s41598-022-06239-y.
18. Skoulidis, F. and J.V. Heymach, 2019. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nat. Rev. Cancer, 19: 495-509.
19. Tsukumo, Y., M. Naito and T. Suzuki, 2020. Influence of EGFR-activating mutations on sensitivity to tyrosine kinase inhibitors in a KRAS mutant non-small cell lung cancer cell line. PLoS ONE, Vol. 15. 10.1371/journal.pone.0229712.
20. Yang, Y.C., K.F. Pan, W.J. Lee, J.H. Chang and P. Tan *et al*., 2020. Circulating proteoglycan endocan mediates EGFR-driven progression of non-small cell lung cancer. Cancer Res., 80: 3292-3304.
21. Zhang, R., J. Zhu, Y. Liu, Y. Xin, Y. Wang, K. Niu and H. Wei, 2021. Efficacy of immune checkpoint inhibitors in the treatment of non-small cell lung cancer patients with different genes mutation: A meta-analysis. Medicine, Vol. 100. 10.1097/MD.0000000000019713.
22. Zhao, Y., H. Wang and C. He, 2021. Drug resistance of targeted therapy for advanced non-small cell lung cancer harbored EGFR mutation: From mechanism analysis to clinical strategy. J. Cancer Res. Clin. Oncol., 147: 3653-3664.
23. Zheng, S., X. Wang, Y. Fu, B. Li and J. Xu *et al*., 2021. Targeted next-generation sequencing for cancer-associated gene mutation and copy number detection in 206 patients with non-small-cell lung cancer. Bioengineered, 12: 791-802.