

Case Report

Unusual Synchronous Multiple Primary Early-Stage Lung Adenocarcinoma with Concomitant MET Exon 14 Skipping, PIK3CA and KRAS Mutations: A Case Report

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Keywords

Synchronous multiple primary lung cancer · Concurrent mutations · Multiple gene mutations · Case report

Abstract

Instruction: Synchronous multiple primary lung cancer (sMPLC) constitutes a distinct subtype of NSCLC, where accurate diagnosis and prognostic evaluation remain challenging. **Case Presentation:** The case involves a 70-year-old male patient admitted to the hospital due to bilateral pulmonary nodules. The patient underwent staged resection. Molecular pathological examination revealed that tumor A harbored concurrent mutations in MET exon 14 skipping and PIK3CA (p.E545K), while tumor B exhibited a KRAS exon 2 (p.G12S/D) mutation. Postoperatively, the patient demonstrated a favorable recovery, with no evidence of recurrence for 1 year. **Conclusion:** This study presents a case of sMPLC in early-stage lung cancer, illustrating the genetic heterogeneity in early-stage lung adenocarcinoma and underscoring the significance of precise evaluation of sMPLC and intrapulmonary metastases.

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Introduction

Based on the 2020 Global Cancer Burden Index analysis released by the World Health Organization's International Agency for Research on Cancer, the leading position of lung cancer in worldwide incidence and mortality was emphasized [1]. With the widespread

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adoption of thin-slice computed tomography in lung cancer screening and the ongoing advancements in clinical research, the detection rate of synchronous multiple primary lung cancers (sMPLCs) has steadily increased [2]. Its characteristic is the occurrence of two or more primary tumors in the lung on the same or opposite side within the same period (i.e., within 6 months). These multifocal lesions are anatomically separate, and their origins are relatively independent [3]. Due to its multiple lesions, scattered distribution, and complex pathological features, sMPLC has garnered extensive clinical attention. Currently, a prevailing challenge with sMPLC is how these nodules are categorized, whether these multiple nodules are independent lesions or intrapulmonary metastases (IPMs), which directly affects therapeutic decisions and prognostic evaluations. Given the significant heterogeneity of lung adenocarcinoma both histologically and molecularly, an integrated histopathological and molecular genetic approach is pivotal to delineate tumor interrelationships in sMPLC, enriching guidelines for staging and treatment [4]. Previous studies have shown that the molecular heterogeneity of sMPLC is related to multiple driver mutation genes such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), proto-oncogene tyrosine-kinase protein (ROS1), mesenchymal epithelial transition factor (MET), and Kirsten rat sarcoma viral oncogene (KRAS). Detecting these high-frequency driver genes in multiple lesions helps to more precisely interpret the clonal origin and tumorigenesis of sMPLC [5–7].

The simultaneous presence of three mutations is exceptionally rare in early-stage NSCLC, and no cases with such genetic co-mutations have been reported. Herein, we report two lesions with similar histopathological characteristics: one nodule harbored a concurrent mutation in MET exon 14 skipping and phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA) (p.E545K), while the other nodule exhibited a KRAS exon 2 (p.G12S/D) mutation. This underscores the genetic heterogeneity in early-stage lung adenocarcinoma and provides a critical basis for the differential diagnosis of sMPLC/IPM. The CARE Checklist has been completed by the authors for this case report and is attached as online supplementary material (for all online suppl. material, see <https://doi.org/10.1159/000540355>).

Case Presentation

A 70-year-old male with a 40-year smoking history presented with chest discomfort, cough, and production of white sputum 3 years ago, without any obvious predisposing factors. He had no significant symptoms of dyspnea, chills, fever, or chest pain. Two months ago, his symptoms worsened. High-resolution computed tomography (HRCT) revealed a ground-glass opacity lesion (tumor A) in the left upper lobe (LUL, 34 × 24 mm) and a solid nodule (tumor B) with irregular high-density shadows in the dorsal segment of the right lower lobe (RLL, 19 × 13 mm) (Fig. 1). There were multiple speckle-like and nodular high-density shadows and calcifications in both lungs. A PET-CT scan showed a hyper-metabolic lesion in the LUL, considering the possibility of malignant lesion. In addition, multiple patchy shadows in the RLL and mild hypermetabolism in bilateral hilar lymph nodes (LNs) were found.

He was admitted to our hospital for further treatment in February 2023. He underwent the first LUL lobectomy with mediastinal LN dissection and the second RLL wedge resection 4 months later. Pathological analysis confirmed both nodules as moderately differentiated invasive adenocarcinoma, without signs of vascular tumor thrombus, perineural invasion, or LN metastasis. Histopathology exhibited that tumor A was acinar and papillary subtype, whereas tumor B was acinar and lepidic subtype (Fig. 2). Molecular pathological analysis using Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) detected multiple gene mutations. The tumor A harbored the double mutation of MET ex14

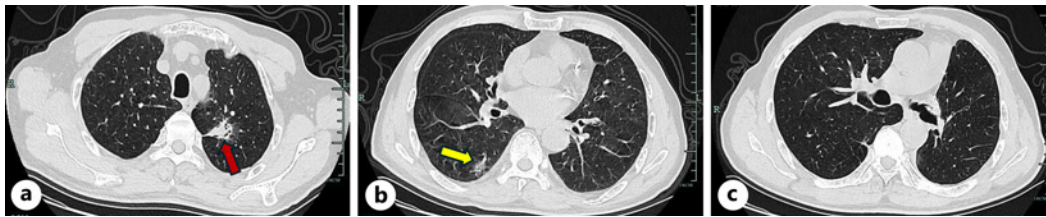


Fig. 1. Computed tomography (CT) images. **a** The red arrow indicated a ground-glass opacity (GGO) lesion in the left upper lobe. **b** The yellow arrow indicated a solid nodule with irregular high-density shadows in the dorsal segment of the right lower lobe. **c** Both lungs showed improved lung markings and increased pulmonary clarity.

skipping and PIK3CA (p.E545K), while the tumor B contained the mutation of the KRAS exon 2 (p.G12S/D) (Fig. 3). Therefore, the 2 lesions were from independent clonal origins. The patient did not receive the MET inhibitor-targeted therapy and recovered well after surgery.

The patient returned to our hospital in October 2023. Follow-up indicated normalized oncologic markers and negative tests for seven lung cancer-specific autoantibodies. HRCT scans revealed improved lung markings and increased pulmonary clarity (Fig. 1). The patient has demonstrated a promising recovery, with no signs of recurrence. Detailed clinicopathological characteristics and Molecular pathological examination are shown in Table 1.

Discussion

Currently, MPLC accounts for an incidence of 2.4%–18.7% in lung cancer, with no significant gender differences, and most patients presenting with dual nodular lesions [8–10]. Over recent years, pathologists have progressively refined their histological understanding of MPLC. Nevertheless, distinguishing between multiple lesions with similar histological types and growth patterns has yet to be elucidated. Accurately differentiating between MPLC and IPM holds significant clinical value since it directly affects tumor staging, therapeutic strategies, and prognosis [9–11]. The Martini and Melamed (MM) criteria, along with the Comprehensive Histologic Assessment (CHA) criteria, serve as primary clinical tools for differentiating between MPLC and IPM by more intricately evaluating the histologic features among multiple lesions [12, 13]. While these methods afford improved differentiation accuracy, relying solely on histological diagnosis in clinical settings has its limitations.

Previous research had underscored the significant genetic heterogeneity of sMPLC with similar histopathological subtypes [14]. Owing to this characteristic, molecular genetics and biology techniques have been increasingly employed in the differential diagnosis between MPLC and IPM. Variability in oncogenic driver gene mutations among multiple nodules indicates distinct clonal origins, whereas intrapulmonary metastatic cancers often display identical mutations. Genomic analyses have ushered in novel strategies for diagnosing sMPLC.

PI3K/AKT/mTOR and RAS/RAF/MEK are two crucial signaling cascades that are activated by RAS to regulate tumorigenesis, progression, and metastasis in lung cancer [15]. Overactivation of these pathways is often due to mutations in high-frequency driver genes such as EGFR, KRAS, BRAF, and PIK3CA [16, 17]. In cases where the pathological subtypes remain consistent, but the relationship between multiple lesions is ambiguous, evaluating the previously mentioned high-frequency driver genes emerges as a crucial differential diagnostic tool [18]. The employed gene testing kit in our case comprised both

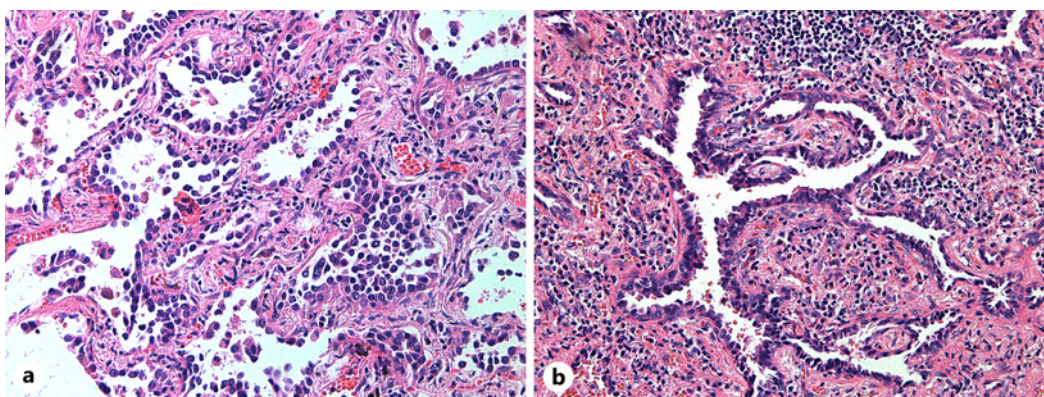
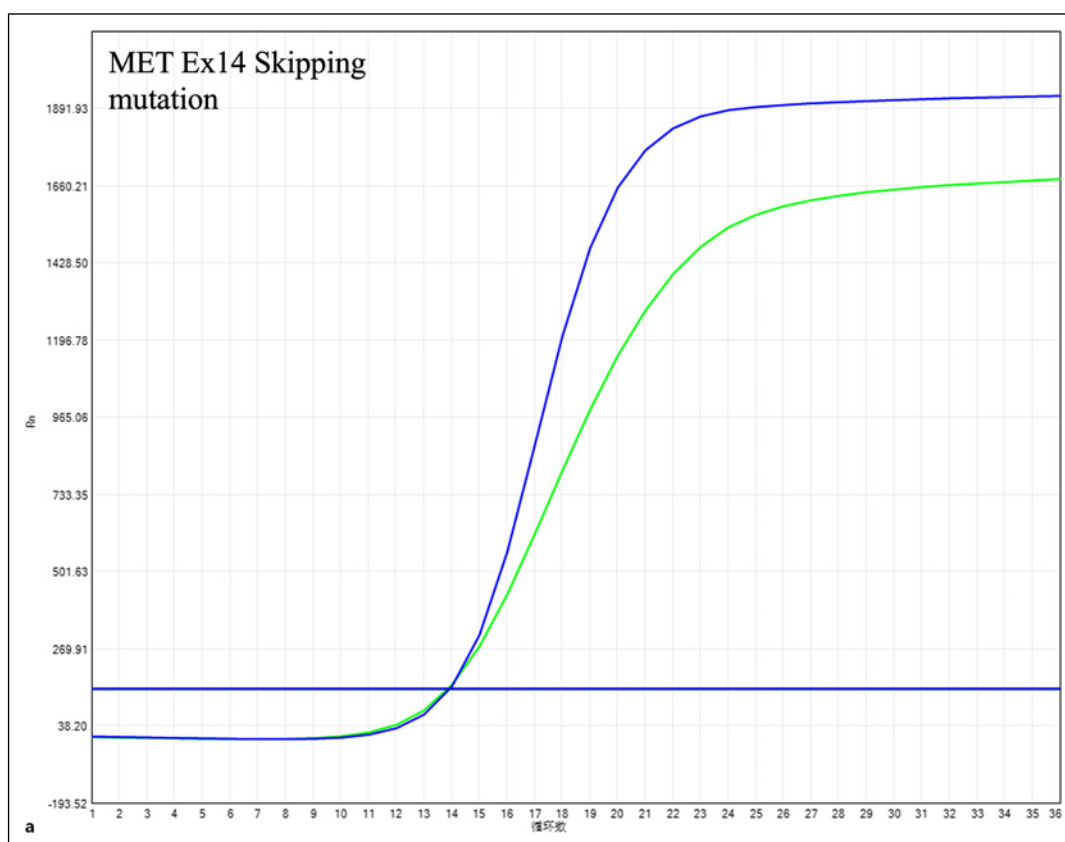


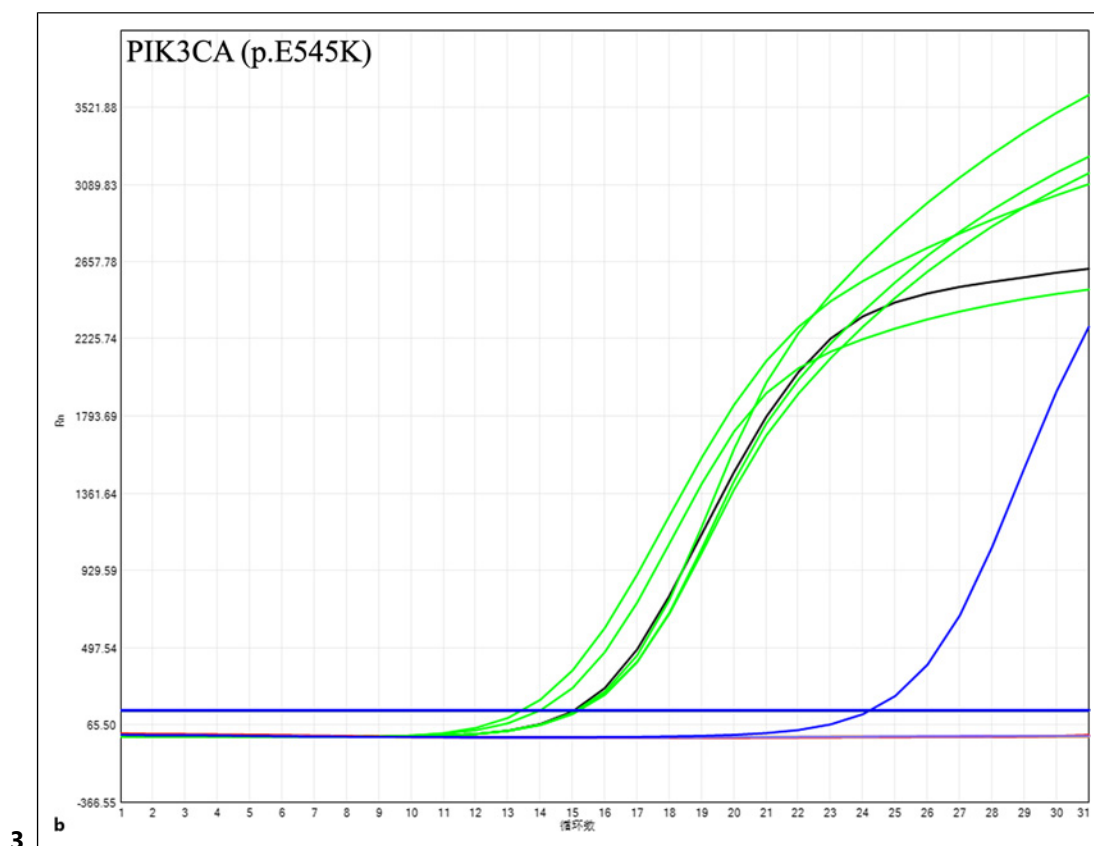
Fig. 2. **a** The mass in the left upper lobe showed acinar and papillary subtype (H&E, ×200). **b** The mass in the right lower lobe showed acinar and lepidic subtype (H&E, ×200).



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vital genes endorsed by national and international guidelines (EGFR/ALK/ROS1) and expanded genes (BRAF/HER2/KRAS/NRAS/PIK3CA/RET/MET) targeting prevalent mutation regions. Intriguingly, genetic diagnostics revealed MET ex14 skipping and PIK3CA dual mutations in tumor A and a KRAS mutation in tumor B. Several driver mutations have been identified in lung adenocarcinomas, most of which occur mutually



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exclusive. Current studies indicate a mutation frequency of only 0.9% for METex14 skipping in lung adenocarcinoma in China, and coexistence with other driver gene mutations is even rarer [19, 20]. Among them, PIK3CA mutations are an exception identified with other driver mutations such as EGFR and KRAS mutations in early-stage NSCLC [21]. However, to our knowledge, this is the first report of simultaneous PIK3CA and MET mutations in a single tumor. Our patient represents the inaugural case report of sMPLC exhibiting three gene mutations between the two lesions in early-stage lung cancer.

For high-risk nodules requiring clinical intervention, surgical resection is the primary therapeutic option in MPLC. The surgical strategy was devised based on the lesion's anatomical location, cardiopulmonary function, and the patient's comprehensive physical status, with priority given to the main lesion [22, 23]. Our patient, an elderly smoker presenting with chest discomfort, cough and expectoration, underwent an exhaustive evaluation. Following this assessment, staged resection of bilateral pulmonary nodules was undertaken to mitigate surgical risks and postoperative complications, thereby optimizing recovery. Given the presence of multiple oncogenic mutations, tumor markers and seven lung cancer-specific autoantibodies were systematically monitored in conjunction with HRCT scans during follow-up. The patient maintained favorable postoperative health, with no evidence of recurrence at the most recent follow-up.

In this case, although the LUL and RLL masses demonstrated analogous histology, they exhibited distinct genetic characteristics. It illustrated that a variety of driver gene mutations can manifest in different lobes concurrently even if early lung cancer. Such intricate mutations

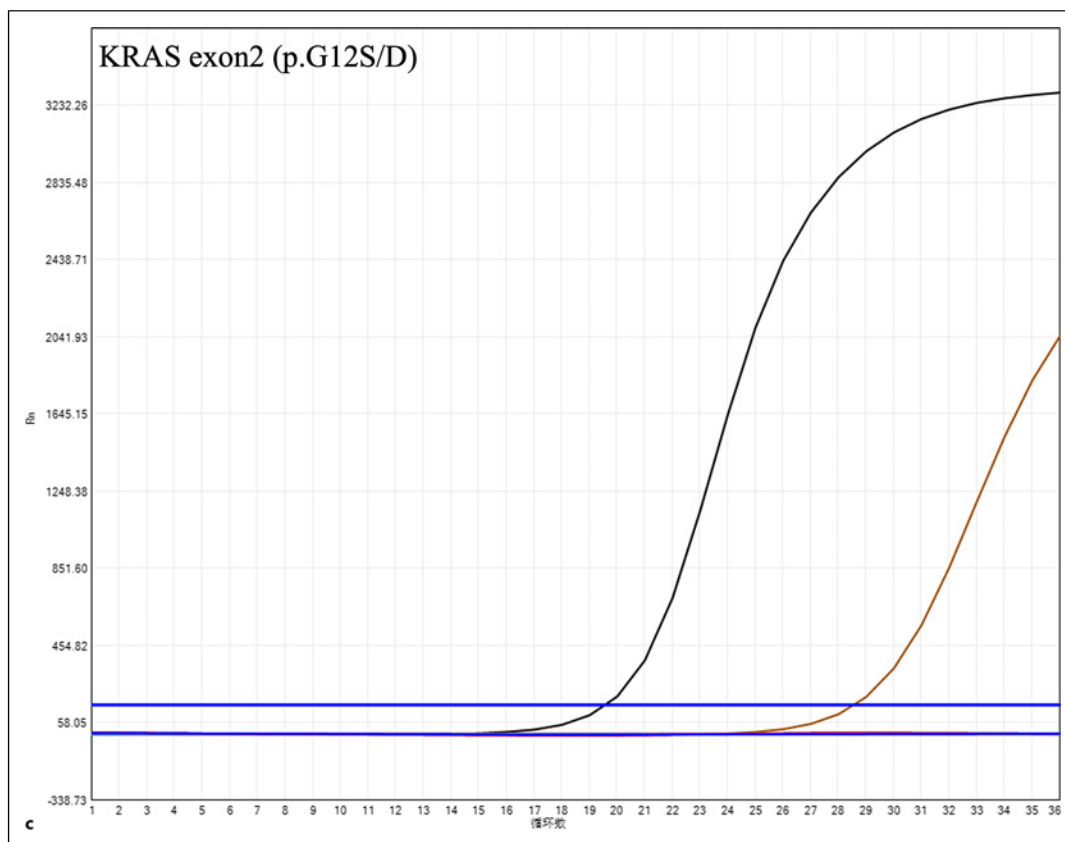


Fig. 3. Molecular pathological examination in multiple pulmonary nodules by ARMS-PCR. MET Ex14 skipping (a) and PIK3CA (p.E545K) mutation (b) testing in the left upper lobe; KRAS exon2 (p.G12S/D) mutation testing in the right lower lobe (c).

Table 1. Clinicopathological characteristics and gene information of sMPLC

	First-stage operation	Second-stage operation
Extent of operation	Lobectomy	Wedge
Location	LUL	RLL
Size	2.4 × 1.5 × 0.7 cm	1.8 × 1.3 × 0.7 cm
Histology	IA	IA
Subtype	Acinal, papillary	Acinal, Lepidic
pTNM stage	T1cN0Mx	T1bN0Mx
Molecular findings	Double mutation of MET ex14 Skipping and PIK3CA	KRAS exon2 p.G12S/D

LUL, left upper lobe; RLL, right lower lobe; IA, invasive adenocarcinoma.

suggest that tumor heterogeneity can be present across distinct tumors simultaneously. Molecular biological analysis provides a more comprehensive foundation for the diagnosis and treatment of such patients. Meanwhile, sMPLC can also be further provided with more abundant genetic data and a whole view of the genetic landscape of the tumors through the application of other molecular detection methods, such as whole exon sequencing and whole genome sequencing.

Conclusion

The case provided diversity of gene expression in early-stage lung adenocarcinoma. As further research into sMPLC is undertaken, incorporating a broader spectrum of genetic molecular features will enable a more precise definition, differentiation, and treatment of MPLC-related challenges, subsequently guiding clinical practice.

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Statement of Ethics

This study protocol was reviewed and the need for approval was waived by the Clinical Research Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine. This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yongjing Zhang was the first author to contribute to this manuscript. Qiqi Gao took part in the investigations and proofreading of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

References

- 1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209–49. <https://doi.org/10.3322/caac.21660>

- 2 Vazquez M, Carter D, Brambilla E, Gazdar A, Noguchi M, Travis WD, et al. Solitary and multiple resected adenocarcinomas after CT screening for lung cancer: histopathologic features and their prognostic implications. *Lung Cancer*. 2009;64(2):148–54. <https://doi.org/10.1016/j.lungcan.2008.08.009>
- 3 Detterbeck FC, Marom EM, Arenberg DA, Franklin WA, Nicholson AG, Travis WD, et al. The IASLC lung cancer staging project: background data and proposals for the application of TNM staging rules to lung cancer presenting as multiple nodules with ground glass or lepidic features or a pneumonic type of involvement in the forthcoming eighth edition of the TNM classification. *J Thorac Oncol*. 2016;11(5):666–80. <https://doi.org/10.1016/j.jtho.2015.12.113>
- 4 Tseng LH, De Marchi F, Pallavajjala A, Rodriguez E, Xian R, Belchis D, et al. Clinical validation of discordant trunk driver mutations in paired primary and metastatic lung cancer specimens. *Am J Clin Pathol*. 2019;152(5):570–81. <https://doi.org/10.1093/ajcp/aqz077>
- 5 Chen K, Chen W, Cai J, Yang F, Lou F, Wang X, et al. Favorable prognosis and high discrepancy of genetic features in surgical patients with multiple primary lung cancers. *J Thorac Cardiovasc Surg*. 2018;155(1):371–9.e1. <https://doi.org/10.1016/j.jtcvs.2017.08.141>
- 6 Zheng R, Shen Q, Mardekian S, Solomides C, Wang ZX, Evans NR 3rd. Molecular profiling of key driver genes improves staging accuracy in multifocal non-small cell lung cancer. *J Thorac Cardiovasc Surg*. 2020;160(2):e71–9. <https://doi.org/10.1016/j.jtcvs.2019.11.126>
- 7 Liu M, He WX, Song N, Yang Y, Zhang P, Jiang GN. Discrepancy of epidermal growth factor receptor mutation in lung adenocarcinoma presenting as multiple ground-glass opacities. *Eur J Cardio Thorac Surg*. 2016;50(5):909–13. <https://doi.org/10.1093/ejcts/ezw113>
- 8 Sun W, Feng L, Yang X, Li L, Liu Y, Lv N, et al. Clonality assessment of multifocal lung adenocarcinoma by pathology evaluation and molecular analysis. *Hum Pathol*. 2018;81:261–71. <https://doi.org/10.1016/j.humpath.2018.01.030>
- 9 Roepman P, Ten Heuvel A, Scheidel KC, Sprong T, Heideman DAM, Seldenrijk KA, et al. Added value of 50-gene panel sequencing to distinguish multiple primary lung cancers from pulmonary metastases: a systematic investigation. *J Mol Diagn*. 2018;20(4):436–45. <https://doi.org/10.1016/j.jmoldx.2018.02.007>
- 10 Mansuet-Lupo A, Barritault M, Alifano M, Janet-Vendroux A, Zarmaev M, Biton J, et al. Proposal for a combined histomolecular algorithm to distinguish multiple primary adenocarcinomas from intrapulmonary metastasis in patients with multiple lung tumors. *J Thorac Oncol*. 2019;14(5):844–56. <https://doi.org/10.1016/j.jtho.2019.01.017>
- 11 Takahashi Y, Shien K, Tomida S, Oda S, Matsubara T, Sato H, et al. Comparative mutational evaluation of multiple lung cancers by multiplex oncogene mutation analysis. *Cancer Sci*. 2018;109(11):3634–42. <https://doi.org/10.1111/cas.13797>
- 12 Martini N, Melamed MR. Multiple primary lung cancers. *J Thorac Cardiovasc Surg*. 1975;70(4):606–12. [https://doi.org/10.1016/s0022-5223\(19\)40289-4](https://doi.org/10.1016/s0022-5223(19)40289-4)
- 13 Girard N, Deshpande C, Lau C, Finley D, Rusch V, Pao W, et al. Comprehensive histologic assessment helps to differentiate multiple lung primary nonsmall cell carcinomas from metastases. *Am J Surg Pathol*. 2009;33(12):1752–64. <https://doi.org/10.1097/PAS.0b013e3181b8cf03>
- 14 Zhou H, Hu Y, Luo R, Zhao Y, Pan H, Ji L, et al. Multi-region exome sequencing reveals the intratumoral heterogeneity of surgically resected small cell lung cancer. *Nat Commun*. 2021;12(1):5431. <https://doi.org/10.1038/s41467-021-25787-x>
- 15 Downward J. Targeting RAS and PI3K in lung cancer. *Nat Med*. 2008;14(12):1315–6. <https://doi.org/10.1038/nm1208-1315>
- 16 Cancer Genome Atlas Research Network. Author Correction: comprehensive molecular profiling of lung adenocarcinoma. *Nature*. 2018;559(7715):E12. <https://doi.org/10.1038/s41586-018-0228-6>
- 17 Sini C, Tuzi A, Rossi G, Russo A, Pezzuto A. Acquired resistance in oncogene-addicted non-small-cell lung cancer. *Future Oncol*. 2018;14(13s):29–40. <https://doi.org/10.2217/fon-2018-0097>
- 18 Higuchi R, Nakagomi T, Goto T, Hirotsu Y, Shikata D, Yokoyama Y, et al. Identification of clonality through genomic profile analysis in multiple lung cancers. *J Clin Med*. 2020;9(2):573–89. <https://doi.org/10.3390/jcm9020573>
- 19 Liu SY, Gou LY, Li AN, Lou NN, Gao HF, Su J, et al. The unique characteristics of MET exon 14 mutation in Chinese patients with NSCLC. *J Thorac Oncol*. 2016;11(9):1503–10. <https://doi.org/10.1016/j.jtho.2016.05.016>
- 20 Awad MM, Oxnard GR, Jackman DM, Savukoski DO, Hall D, Shivdasani P, et al. MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and c-met overexpression. *J Clin Oncol*. 2016;34(7):721–30. <https://doi.org/10.1200/JCO.2015.63.4600>
- 21 Bar J, Damianovich M, Hout SG, Dar E, Cohen Y, Perelman M, et al. Genetic mutation screen in early non-small-cell lung cancer (NSCLC) specimens. *Clin Lung Cancer*. 2014;15(2):159–65. <https://doi.org/10.1016/j.clcc.2013.11.005>
- 22 Pezzuto A, Trabalza Marinucci B, Ricci A, Ciccozzi M, Tonini G, D'Ascanio M, et al. Predictors of respiratory failure after thoracic surgery: a retrospective cohort study with comparison between lobar and sub-lobar resection. *J Int Med Res*. 2022;50(6):3000605221094531. <https://doi.org/10.1177/03000605221094531>
- 23 Jung EJ, Lee JH, Jeon K, Koh WJ, Suh GY, Chung MP, et al. Treatment outcomes for patients with synchronous multiple primary non-small cell lung cancer. *Lung Cancer*. 2011;73(2):237–42. <https://doi.org/10.1016/j.lungcan.2010.11.008>