Primary lung adenocarcinoma with breast metastasis harboring the EML4-ALK fusion: A case report

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Abstract. Pulmonary adenocarcinoma with breast metastasis is rarely encountered in clinical practice. Therefore, precise clinical diagnosis of patients with this disease is crucial when selecting subsequent treatment modalities and for overall prognosis assessment. The present study reported on a case of lung cancer with breast metastasis harboring the EML4-ALK fusion. The patient was initially diagnosed with triple-negative breast cancer with lung metastasis, but comprehensive breast cancer treatment was ineffective. Reevaluation of the patient's condition via lung biopsy revealed primary lung adenocarcinoma. In addition, the results of genetic testing revealed the EML4-ALK fusion protein in both lung and breast tissues. After treatment with ALK inhibitors, the patient's symptoms improved rapidly. This case highlights the prolonged diagnostic journey from presentation with a breast mass to ultimately being diagnosed with lung cancer with breast metastasis, underscoring the critical need for heightened awareness among clinicians regarding the possibility of rare metastatic patterns. Timely identification of lung cancer with breast metastasis, facilitated by comprehensive genetic testing, not only refines treatment decisions but also emphasizes the importance of interdisciplinary collaboration in navigating complex clinical scenarios. Such insight contributes to the ongoing development of personalized cancer care that guides clinicians toward more effective and tailored therapeutic strategies for patients with similar diagnostic challenges.

Introduction

According to American Cancer Society estimates of cancer data, primary lung cancer ranked second in incidence and first in mortality in 2023, with distant metastasis being a major contributor to the high fatality rate (1). Lung cancer commonly metastasizes to the brain, bones, adrenal glands and liver; however, metastasis in the breast is relatively uncommon. According to clinical and autopsy findings (2), only 0.5-1.2 and 1.7-6.6% of malignant tumors metastasize to the breast, respectively. Given that breast cancer is the most common cancer type in women and considering the lack of specific features in imaging examinations that can be used to distinguish primary from metastatic tumors (3), patients with lung cancer breast metastasis are often misdiagnosed as having primary breast cancer, leading to erroneous treatment strategies. Immunohistochemistry (IHC) combined with next-generation sequencing (NGS) can increase diagnostic accuracy and provide crucial insight into disease treatment and etiology. Furthermore, NGS has become a powerful tool for diagnosis in challenging cases (4).

The rate of anaplastic lymphoma kinase (ALK) gene mutation in primary lung cancer is ~3-5%, with the EMAP like 4 (EML4)-ALK fusion being the most common anomaly. ALK is an important target for targeted lung cancer therapy. With ongoing research into the molecular mechanisms of tumors and advancements in clinical trials of targeted drugs, various ALK tyrosine kinase inhibitors (TKIs) have proven to be effective treatments for patients with ALK fusion-positive lung cancer; these treatments have provided significant clinical benefits (5-7).

The present study reported on a rare case in which a patient was diagnosed with primary lung adenocarcinoma with breast metastasis through IHC and genomic analysis. The EML4-ALK fusion was confirmed, and ALK TKI treatment prolonged patient survival. With this case, challenges in accurate diagnosis and treatment were encountered and showcased. Due to the low frequency of breast metastasis in patients with lung cancer and the potential for misdiagnosis, imaging and IHC have certain limitations. Comprehensive genomic testing holds significant importance for identifying effective therapeutic strategies for patients, which can extend progression-free survival, alleviate symptoms and improve the overall quality of life. Furthermore, the present findings
provide evidence that shows the effectiveness of ALK TKIs in the treatment of patients with EML4-ALK fusion-positive lung cancer. The outcome for this patient suggests the need for more targeted and personalized therapeutic approaches in similar cases.

Case report

A 42-year-old non-smoking female sought medical attention at the Affiliated Hospital of Zunyi Medical University (Zunyi, China) in March 2020 due to a palpable and painful lump in the left breast. Breast MRI revealed multiple nodules in the left breast and axilla. Pathologists described the microscopic characteristics of the H&E-stained sections as follows: Tubular structures were observed; the nuclei of the cancer cells were generally enlarged, with variability in size and shape, including the formation of small nucleoli; and mitotic figures were present. According to the Nottingham grading system (8), the evaluation indicated 3 points for tubular formation, 2 points for nuclear grade and 1 point for mitotic count, resulting in a total of 6 points, indicating grade II (moderately differentiated). IHC (9) was performed on a BOND-MAX Fully Automated IHC and ISH Staining System (Leica Microsystems, GmbH). The ready-to-use primary antibodies, including CK5/6 (cat. no. GT243802), P120 (cat. no. GT209902), CK7 (cat. no. GT244602), E-cadherin (cat. no. GT210702), TTF-1 (cat. no. GT218002), Ki-67 (cat. no. GM724002) and p63 (cat. no. GT253202), were all purchased from Gene Tech Co., Ltd. The secondary antibody and the chromogenic system were included as part of the instrument's kit. IHC revealed negative expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER)2 and cytokeratin (CK)5/6, but positive expression of P120, CK7, E-cadherin, thyroid transcription factor (TTF)-1 and Ki-67; there was also no p63 expression in tumor-associated myoepithelial cells (Fig. 1A-F). No evidence of metastasis was found in the lymph nodes. The pathologist was initially unaware of the lung nodules, the histological features of the breast specimens were consistent with those of primary breast cancer and TTF-1 positivity was not a concern among the pathologists. Subsequent imaging studies, including chest CT and brain MRI, revealed nodules in the lungs and intracranial regions (Fig. 2A-C). However, the pathologists were not able to distinguish between metastatic breast carcinoma and primary breast carcinoma morphologically. Then, considering the absence of respiratory symptoms in the patient, the greater number and size of breast nodules compared to lung nodules and the 40% occurrence rate of lung metastases in triple-negative breast cancer patients (10), clinicians ultimately diagnosed the patient with triple-negative infiltrating breast cancer (T1N0M1, stage IV) with pulmonary and intracranial metastases following discussions among pathologists and clinicians. Systemic chemotherapy was recommended, but the patient declined and opted for an alternative Traditional Chinese Medicine-based antitumor treatment, which the patient self-administered (details not disclosed).

In August 2020, the patient experienced aggravated symptoms, including lower back pain, without cough. Chest and abdominal CT scans, along with cranial MRI, indicated enlargement of the lesions when compared to previous assessments (Fig. 3A and B). The newly identified metastatic sites included the 8th thoracic vertebra and the 4th lumbar vertebra (Fig. S1A and B), as well as liver metastases (Fig. 3C). Breast ultrasound revealed enlargement of the left breast nodule and emergence of a new nodule in the right breast. Considering the progression of the disease, the patient was referred to the Second Affiliated Hospital of Zunyi Medical University (Zunyi, China). Based on the breast pathology results, following the guidelines for stage IV breast cancer, a salvage chemotherapy regimen comprising taxanes and anthracyclines was initiated [intravenous paclitaxel (albumin-bound) 400 mg + epirubicin 120 mg]. Palliative radiotherapy was administered to alleviate symptoms at the metastatic lesion in the 4th lumbar vertebra.

After the second round of chemotherapy in October 2020, the patient was reevaluated using imaging. The breast and pulmonary lesions showed no significant changes, but increases in the size and number of metastatic tumors were observed in the intracranial and hepatic regions (Fig. 3D-F). A new metastatic lesion in the left scapula was also identified (Fig. S2). Treatment response was assessed and determined as progressive disease according to the Response Evaluation Criteria in Solid Tumors criteria 1.1 (11). Concurrently, the patient experienced severe headaches, prompting palliative whole-brain radiotherapy. Due to the ineffectiveness of breast cancer treatment regimens, the possibility of primary lung cancer with breast metastasis was suspected, and local anesthesia-assisted biopsy of the right lower lung nodule was performed. After examination of the histological features of the specimen, the pathologists were inclined to diagnose the patient with primary lung adenocarcinoma. The ready-to-use antibody reagents for GDFPF-15 (cat. no. GT204902), GATA3 (cat. no. GT218702) and Napsin-A (cat. no. GT218502) were also purchased from Gene Tech Co., Ltd. IHC results indicated positivity for CK7, GCDFP-15 (cat. no. GT204902), GATA3 (cat. no. GT218702) and Napsin-A, and negativity for TTF-1 and Napsin-A, ER, PR, HER2, gross cystic disease fluid protein 15 (GDFPF-15) and GATA binding protein 3 (GATA3) (Fig. 4A-G). Further lung tissue detection assays were performed using the Multi-Mutation Gene Diagnostic Kit (Amoy Diagnostics, Co., Ltd.). Using ADx-ARMS® technology, lung tissue DNA was analyzed for EGFR mutations, and ALK and ROS1 gene fusions were evaluated in RNA samples via reverse transcription PCR (12). The results revealed an EML4-ALK mutation, but the specific fusion type could not be determined. This evidence suggested that the lung was the primary site, and clarification was needed to determine whether the breast lesion was primary or metastatic. Previous breast tissue samples were sent to the College of American Pathologists and Clinical Laboratory Improvements Amendments accredited central laboratory at Nanjing Geneseeq Technology, Inc. for analysis. A customized xGen lockdown probe panel (Integrated DNA Technologies) was used for targeted enrichment of 425 predefined genes. The enriched libraries were sequenced on HiSeq 4000 NGS platforms (Illumina, Inc.) (13) to coverage depths of at least x100 and x300 after removing PCR duplicates for tumor and normal tissue, respectively. The results confirmed EML4-ALK fusion (E18:A20). In addition, mutations in breast cancer susceptibility genes, including GATA3, BRCA1, BRCA2, tumor protein 53 and partner and localizer of BRCA2 (PALB2), were negative. Furthermore, the pathologist observed no microacinar
structures or signet ring tumor cells in the histology images of the breast and lung tissue specimens (Figs. 1A and 4A). The combined IHC and genetic test results for both the lung and breast tissue samples were comprehensively assessed and it was determined that the patient had right lower lung adenocarcinoma T1bN3M1c-stage IVB (according to the 8th edition of the American Joint Committee on Cancer staging manual) (14) with the EML4-ALK fusion. The subsequent treatment plan involved ALK-TKI targeted therapy.

Oral treatment with crizotinib capsules [250 mg per os (PO) twice daily] was initiated in November 2020, and remarkably, this targeted therapy demonstrated significant efficacy. The primary lung lesion and other metastatic lesions consistently decreased in size at 6 months post-treatment initiation (Fig. 5A-F). However, after 9 months of targeted therapy, there was an indication of increased lesions in the brain, although no clinical symptoms were reported. The right lower lobe nodule continued to shrink and the other lesions remained stable in size (Fig. 5G-I). During treatment, the patient's quality of life was assessed using a scale developed by the European Organisation for Research and Treatment of Cancer (EORTC), the EORTC QLO-C30 (V3.0) (15). This tool, known for its reliability and validity, covers the essential aspects of health-related quality of life and is suitable for all cancer patients. The results indicated that during the 9 months of crizotinib treatment, the patients’ quality of life improved compared to that during the radiotherapy and chemotherapy periods. Specifically, improvements were observed in physical functioning (daily activities, walking, etc.), role functioning, emotional functioning (anxiety, worry, irritability, depression), social functioning and overall health status. However, no significant differences were observed in terms of fatigue, pain or nausea/vomiting. After approximately one year of treatment, the patient's condition deteriorated and she presented with bilateral lower limb numbness and motor abnormalities. MRI revealed multiple new metastases in the cervical and thoracic spinal cord (Fig. 6A-C). Despite no significant changes in the primary lung lesion or other metastatic lesions, disease progression was considered. In November 2021, the patient commenced oral treatment with second-generation ALK-TKI
Figure 3. Radiological images of the patient before and after chemoradiotherapy. Before chemotherapy in August 2020: (A) Chest CT image showing the larger nodule, measuring ~15x13 mm and located in the right lower lung lobe (arrow); this finding was similar to that observed in the patient's scans from March 2020. (B) Brain MR image showing a 17x19-mm round lesion in the left frontal lobe (arrow). (C) Abdominal CT image revealing a newly developed round low-density lesion in the left lobe of the liver (arrow) measuring ~16 mm. After chemotherapy in October 2020: (D) Chest CT image showing the nodule in the right lower lobe of the lung (arrow) showed no changes compared to that before therapy. (E) Brain MR image showing the lesion in the left frontal lobe (arrow) was slightly smaller than before, with surrounding edema. (F) Abdominal CT image showing multiple liver lesions of varying sizes and round shapes (arrow), with the larger lesion measuring ~20 mm, and increasing in number and size compared to those before chemotherapy. CT, computed tomography; MR, magnetic resonance.

Figure 4. Pathology and immunohistochemical findings of lung nodule biopsy samples. (A) H&E staining revealed a primary adenocarcinoma. (B-G) IHC for (B) progesterone receptor, (C) estrogen receptor, (D) human epidermal growth factor receptor-2, (E) gross cystic disease fluid protein 15, (F) GATA binding protein 3 and (G) Napsin-A; all results were negative. (H) IHC for thyroid transcription factor-1 revealed strong nuclear positivity. (I) IHC for cytokeratin-7 showed strong cytoplasmic positivity. The magnification is x200 in all images (scale bars, 50 µm). IHC, immunohistochemistry.
ceritinib capsules (450 mg PO once a day) for targeted therapy. Palliative radiotherapy was administered to the cervical spine and lumbar vertebrae. During radiotherapy, the patient's quality of life gradually decreased, mainly manifested as decreases in physical functioning (sensory disturbances in both lower limbs, urinary and fecal incontinence) and overall health status (Grade III bone marrow suppression, fatigue and severe pain). Consequently, radiotherapy was temporarily stopped and the patient was discharged to continue oral ceritinib treatment. Subsequent telephone follow-ups were performed twice, at 1
and 2 months after discharge, with a total survival period of 22 months and 26 days (the starting point was the date of the patient's first visit to the hospital).

Discussion

Primary lung adenocarcinoma is a common malignancy with a high mortality rate, and the occurrence of distant metastasis is the most critical factor that impacts clinical treatment and prognosis. Breast metastasis is uncommon in lung adenocarcinoma, and the incidence of metastasis in the breast from non-mammary sources is estimated to not exceed 2% (16). Accurately distinguishing between primary breast cancer and metastatic breast cancer poses a significant challenge.

A clear distinction between primary and secondary tumors is crucial for clinical treatment decision-making and prognostic assessment. Differential diagnosis is challenging to achieve through imaging methods alone, but IHC provides valuable insight. In previous case reports (17-19), IHC was commonly used to determine the tumor origin, which is consistent with the initial diagnostic approach for the patient of the present study. TTF-1 and Napsin-A are robust biological markers for lung adenocarcinoma; they are expressed in ~80% of cases but are rarely expressed in other cancers. The combined use of TTF-1 and Napsin-A promotes the accurate identification of metastatic adenocarcinoma originating from the lungs. HER2, ER, PR, GATA3 and GCDFP-15 are key and characteristic IHC markers for breast cancer. ER and PR are expressed in ~80 and 60% of primary breast cancers, respectively (20). GATA3 and GCDFP-15 are recently confirmed markers with high sensitivity in primary breast cancer and are expressed in 67-95 and 60% of cases, respectively (21). These markers are rarely expressed in lung adenocarcinoma and can, to a certain extent, assist in making a diagnosis and differential diagnosis.

However, IHC results lack 100% sensitivity and specificity, and although rare, TTF-1 positivity can occur in patients with primary breast cancer (22). Of note, there are certain differences between the present case and previously reported cases. First, Wang et al (23) integrated data of 7 patients with primary lung cancer breast metastasis and proposed that the combination of TTF-1 and Napsin-A can provide the greatest benefit in clinical practice, suggesting that metastatic breast nodules typically present unilaterally without pain. Ji et al (24) reported two cases of lung adenocarcinoma breast metastasis in which the diagnosis primarily relied on medical history and IHC results, and both patients had a history of lung cancer. In the case of the current study, the patient presented with painful breast nodules as the initial symptom, with breast nodules larger than the pulmonary lesion and no history of lung cancer, thus leading to clinicians' confusion. In addition, Ali et al (25) analyzed 12 cases of non-small cell lung cancer (NSCLC) breast metastasis, 5 of which were initially misinterpreted by pathologists as primary breast cancer (PBC). Distinguishing poorly differentiated lung adenocarcinoma from triple-negative PBC is morphologically challenging, as indicated by their study. Initially, the breast specimen in the present case suggested moderately differentiated adenocarcinoma, and upon learning about the patient's pulmonary nodules, pathologists should have been alerted, prompting further testing for GCDFP-15 or GATA3, as these markers may indeed be expressed in PBC. Furthermore, relying solely on TTF-1 results lacks a certain degree of reliability. In the present case, due to the negative expression of Napsin-A in the lung specimen and the positive expression of TTF-1, in combination with the fact that triple-negative breast cancer is more prone to metastasis compared to other types of breast cancer, it may be reasoned that relying solely on TTF-1 positivity is insufficient to support the diagnosis of primary lung cancer metastasizing to the breast. Initially, chemotherapy with palliative taxel was employed, which is also a frontline chemotherapy drug for lung adenocarcinoma. However, after 2 cycles of treatment, the pulmonary nodules did not shrink, adding to the clinical confusion in terms of diagnosis and treatment.

Genetic testing can assist in addressing this dilemma; it may be used to understand whether a patient carries susceptibility gene mutations for cancer to help confirm diagnostic suspicions and to determine whether the tumor may be sensitive to certain drugs, thus further guiding precise treatment and extending patient survival. As demonstrated in the present case, conducting additional genetic testing on both breast and lung tissue samples did not only provide useful information regarding the ALK mutation, allowing the patient to benefit from targeted therapy, but also demonstrated that the patient tested negative for susceptibility gene mutations associated with breast cancer. This method further strengthens our ability to accurately diagnose patients and guide anticancer treatment. Of note, as NGS is rarely used for primary breast cancer and previous reports seldom utilize NGS to aid in diagnosis, the breast biopsy specimen was not immediately sent for genetic testing at initial diagnosis. However, in similar cases in the future, when the patient is unwilling to clarify the nature of lung lesions, sending breast specimens for NGS may provide more treatment options for patients, leading to prolonged survival. In the future, if a patient presents with both lung and breast lesions, particularly if the breast tumor is triple-negative, regardless of the size of the lesion or the presence of respiratory symptoms, further clarifying the nature of the lung lesion is advisable. If the patient is unwilling to clarify the nature of the lung lesions, breast specimens should be sent for NGS. This approach may offer patients more treatment options and reduce the risk of misdiagnosis.

In the present study, the ARMS-PCR method was utilized to analyze lung specimens and the results revealed the EML4-ALK fusion. Unfortunately, due to the use of PCR for testing lung tissue specimens, it was not possible to determine the type of EML4-ALK variant. With respect to the breast biopsy specimens, 425-panel NGS was performed and the results indicated that the EML4-ALK fusion gene was present (E18:A20). The EML4-ALK fusion gene has been conclusively identified as a characteristic gene in NSCLC. In a study by Fukuyoshi et al (26), 90 patients with breast cancer were assessed, although the EML4-ALK fusion was not detected. Another study (27) reported that in comprehensive genomic analyses, ALK fusions/rearrangements were identified in ~0.5-0.8% of cancers (28,29). Specifically, among patients with NSCLC, the prevalence of ALK fusions/rearrangements exceeds 3%, while the frequency of ALK fusions/rearrangements in non-NSCLC tumors is ~0.2%. This finding suggests that ALK fusions/rearrangements are rare in breast cancer. The positive ALK result in the present case strongly supported the
hypothosis that the primary tumor was NSCLC. Furthermore, genetic analysis of the breast specimen revealed negativity for mutations in breast cancer susceptibility genes such as BRCA1, BRCA2, TP53 and PALB2, thus providing further evidence that the breast lesion was a metastatic deposit from the primary lung adenocarcinoma rather than a dual-origin tumor (30-32).

In terms of treatment, the ALK fusion protein serves as a crucial target for molecular targeted therapy in lung cancer, with the EML4-ALK fusion being the predominant fusion type, constituting 90-95% of all ALK fusions. There are eight variants of the EML4-ALK fusion protein that are classified into ‘long’ and ‘short’ types, with protein stability being the primary biological distinction; these variants ultimately lead to varying responses to ALK TKIs (33,34). Clinical trial results suggest that ALK TKIs are most effective against tumors harboring the V2 variant fusion, while tumors harboring the V3 variant fusion exhibit a shorter duration of response to ALK TKIs. The E18:A20 variant identified in the patient described herein was categorized as the V5 subtype, representing 1.56% of all variants (35,36). Currently, there is no established evidence regarding the potential benefits of ALK-TKI treatment for tumors with the V5 variant fusion. In the present case, the patient was treated with the first-generation ALK-TKI crizotinib and exhibited one-year progression-free survival. This outcome may provide insight into clinical treatment and targeted drug selection for this specific type of mutation.

In conclusion, breast metastatic carcinoma of non-mammary origin is rare and is prone to misdiagnosis and oversight in clinical settings. Vigilance should be maintained for such patients, particularly when encountering situations similar to those experienced by the patient described herein. The patient in this case sought medical attention with a chief complaint of a breast lump, posing a diagnostic challenge because there were no apparent respiratory symptoms. Both the breast and lung lesions exhibited multiple nodules of comparable size, complicating the precision of the diagnosis. In the initial assessment, emphasis should be placed on distinguishing between the primary lesion and metastatic lesions. In addition, heightened attention should be directed toward the application of genetic testing technologies, which promote accurate diagnoses, personalized precision medicine in clinical practice and ultimately prolong patient survival.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The present manuscript contains original data generated using NGS, which may be found in the Sequence Read Archive (SRA) under accession no. SRR28475470 or at the following URL: https://www.ncbi.nlm.nih.gov/sra/SRR28475470.

Authors' contributions

SX and YB were primarily responsible for the study and contributed to its conception and design. WZ and YZ obtained and analyzed the patient information and contributed to manuscript drafting and critical revision of the intellectual content. LZ performed the analysis and interpretation of the CT and MRI data. NT performed the histological examination of the tumor. WZ, YZ, LZ, NT, YB and SX confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was conducted at the Second Affiliated Hospital of Zunyi Medical University and was approved by the Institutional Ethics Review Board (Zunyi, China; approval no. KYLL-2023-032). The patient voluntarily agreed to participate in the study and provided written informed consent, which included the publication of this case report.

Patient consent for publication

Written informed consent for publication of the article, including clinical data and images, was obtained from the patient.

Competing interests

The authors declare that they have no competing interests.

References


