A Case of Large-Cell Neuroendocrine Carcinoma Harboring an EML4–ALK Rearrangement with Resistance to the ALK Inhibitor Crizotinib

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CASE REPORT

A 43-year-old never-smoking woman presented with a palpable mass in the left breast. Chest radiography and computed tomography revealed a 5-cm solitary tumor in the left upper lung field (Fig. 1*A*, *B*) and multiple mediastinal lymph node enlargements in addition to the breast tumor. Contrast-enhanced brain magnetic resonance imaging revealed multiple, asymptomatic metastases in the brain (Fig. 1*C*) and a skin metastasis in the scalp. Bone metastasis in the pelvis and hepatic metastasis were also observed. The resected breast sections revealed malignant neoplasia organized into either solid nests or trabeculae of tumor cells with necrotic foci and rosette-like structures (Fig. 2*A*). Tumor cells showed moderately abundant cytoplasm, pleomorphic and vesicular nuclei, and mitosis (up to 20 mitoses/2 mm^2). Immunohistochemical analysis showed that the tumor cells were diffusely and strongly positive for cytokeratin (CK) 7, pan-CK, and neuroendocrine markers (chromogranin, synaptophysin [Fig. 2*B*], and CD56) and negative for thyroid transcription factor-1, estrogen receptor, progesterone receptor, CK5/6, and CK20. Breast tumor specimen analysis before treatment indicated a malignant neoplasm with neuroendocrine structure that was positive for neuroendocrine markers, leading to a diagnosis of a large-cell neuroendocrine carcinoma (LCNEC) with suspected metastasis. Transbronchial biopsy of the lung tumor before treatment showed pathological and immunohistochemical findings similar to the findings for breast tumor. Tumor cells from the lung, breast, and skin were diffusely and strongly positive for anaplastic lymphoma kinase (ALK) by immunohistochemistry using rabbit monoclonal antibody (D5F3; Cell Signaling Technology, Danvers, MA) (Fig. 2*C*). Fluorescence in situ hybridization analysis with break-apart probes for the ALK gene indicated the presence of an ALK rearrangement in the breast (Fig. 2*D*) and lung tumors and skin metastasis. Multiplex reverse transcriptase–polymerase chain reaction revealed amplification of echinoderm microtubule–associated protein-like 4 (EML4)-ALK variant 2 (E20; A20) in the breast tumor. Clinically, the tumors were diagnosed as primary lung carcinoma with metastases, including metastasis to the breast. The patient was treated with crizotinib, the first clinically available ALK tyrosine kinase inhibitor, and the lung tumors remained stable with treatment. Six weeks later, enhanced brain magnetic resonance imaging revealed marked increase in the size of the right cerebellar and left temporal lesions (Fig. 1*D*) and progression of the skin metastasis, indicating both resistance of the tumors to crizotinib and progressive disease.

DISCUSSION

The case presented here is thought to be the first report of an LCNEC harboring an EML4–ALK rearrangement that also showed resistance to the ALK inhibitor crizotinib. In a subset of non–small-cell lung carcinoma, rearrangement of the EML4 and ALK genes creates the oncogenic fusion gene EML4–ALK, which encodes a chimeric protein with constitutive kinase activity that is most commonly seen in adenocarcinoma[1–3] and has been reported in two previous cases of small-cell carcinoma.[4,5] However, neither ALK rearrangement nor amplification have been previously observed in LCNEC.[6] Wong et al.[2] reported that two of 13 cases harboring EML4–ALK rearrangements had unusual histological features representing squamous and glandular components. Therefore, some combined carcinomas comprising adenocarcinoma and nonadenocarcinoma components may show ALK arrangement. In this case, the primary lung tumor may have been a combination of LCNEC and adenocarcinoma. Clinical trials report that the ALK inhibitor crizotinib is highly effective for patients with the ALK rearrangement.[7] However, as the tumor in this case was resistant to crizotinib, ALK rearrangement may not be of practical importance in LCNEC, and neuroendocrine tumors harboring this rearrangement may be less responsive to ALK inhibitors.

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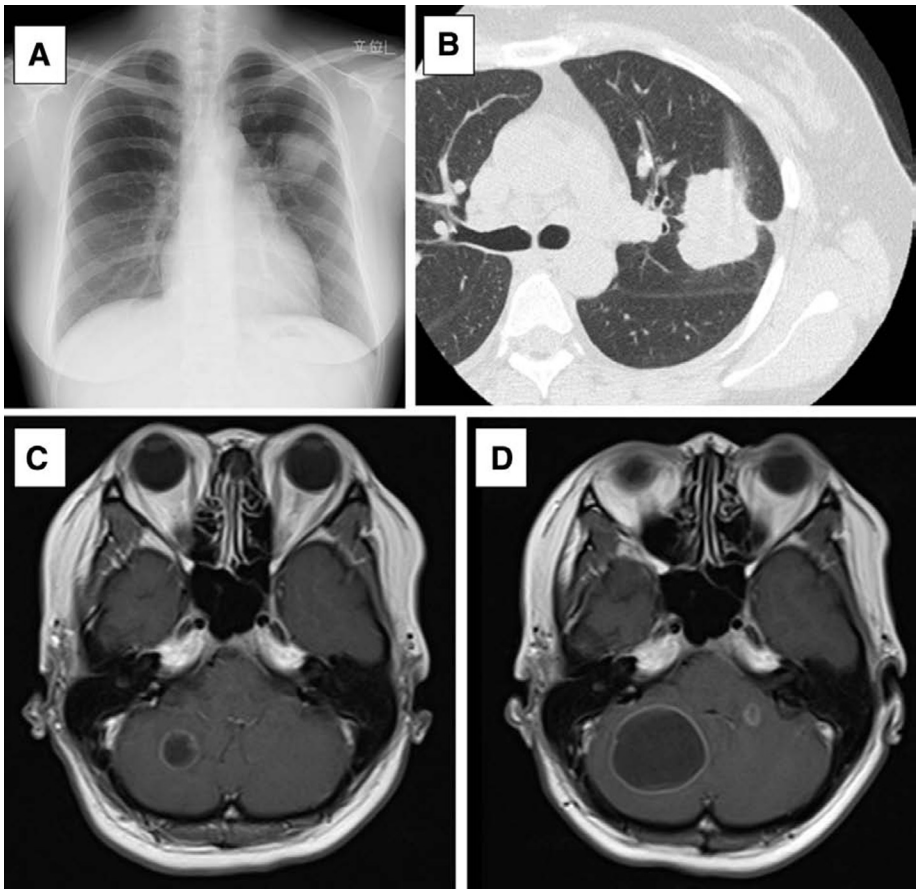

FIGURE 1. Imaging findings. *A,* Chest radiograph on admission showing a mass in the left upper field; (*B*) chest computed tomography scan on admission showing a lung mass in the left upper lobe; (*C*) enhanced brain MRI scan on admission showing metastasis to the right cerebellar region; (*D*) subsequent enhanced brain MRI revealing an increase in the size of the right cerebellar lesion. MRI, magnetic resonance imaging.

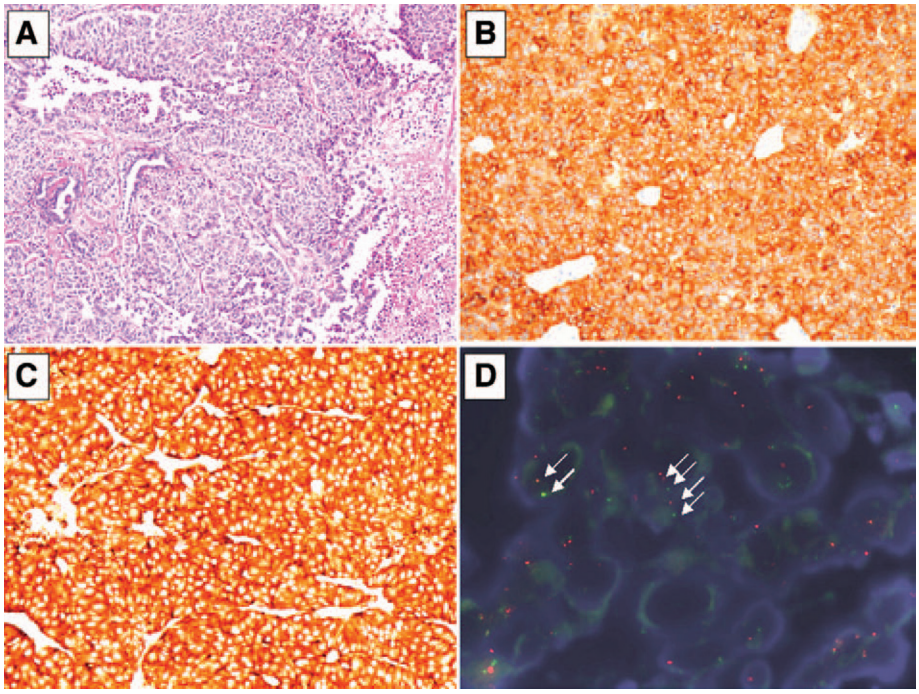

FIGURE 2. Histopathological findings of the breast. *A,* Low-power image of a hematoxylin and eosin–stained section from the breast tumor showing cells organized in solid nests or forming trabeculae with foci of necrosis and rosette-like structures; (*B*) immunohistochemical analysis of the breast tumor showing strong diffuse synaptophysin positivity; (*C*) immunohistochemical analysis showing strong diffuse ALK positivity; (*D*) fluorescence in situ hybridization analysis of the ALK locus using a break-apart probe strategy. Approximately 58% of breast tumor cells showed rearrangement at the ALK locus, as demonstrated by split red/green signals (arrows). ALK, anaplastic lymphoma kinase.

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REFERENCES

1. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–566.
2. Wong DW, Leung EL, So KK, et al.; University of Hong Kong Lung Cancer Study Group. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer* 2009;115:1723–1733.
3. Takeuchi K, Soda M, Ishikawa Y, et al. RET, ROS1 and ALK fusions in lung cancer. *Nat Med* 2012;18:378–381.
4. Toyokawa G, Takenoyama M, Taguchi K, et al. An extremely rare case of small-cell lung cancer harboring variant 2 of the EML4-ALK fusion gene. *Lung Cancer* 2013;81:487–490.
5. Toyokawa G, Taguchi K, Ohba T, et al. First case of combined small-cell lung cancer with adenocarcinoma harboring EML4-ALK fusion and an exon 19 EGFR mutation in each histological component. *J Thorac Oncol* 2012;7:e39–e41.
6. Nakamura H, Tsuta K, Yoshida A, et al. Aberrant anaplastic lymphoma kinase expression in high-grade pulmonary neuroendocrine carcinoma. *J Clin Pathol* 2013;66:705–707.
7. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363:1693–1703.

