Pulmonary Epithelial–Myoepithelial Carcinoma Without AKT1, HRAS or PIK3CA Mutations: A case report

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Case Report

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Abstract

Background: Pulmonary epithelial–myoepithelial carcinoma is a rare subtype of lung cancer. Because of its rarity, the molecular information on this carcinoma is insufficient.

Case presentation: We report a case of pulmonary epithelial–myoepithelial carcinoma without *AKT1*, *HRAS* or *PIK3CA* mutations in a 76-year-old woman. Computed tomography revealed a tumor located in the left lower lung. Thoracoscopic left lower lobectomy was performed. Histopathologically, the tumor consisted of duct-like structures and polygonal-to-spindle-cell features. The duct-like structures were composed of two distinct cell layers. Immunohistochemically, the inner layer consisted of cuboidal cells that were positive for pan-cytokeratin and negative for p63, whereas the outer layer consisted of polygonal and spindle cells that were positive for p63 and weakly positive for pan-cytokeratin. We evaluated mutations in *AKT1*, *BRAF*, *CTNNB1*, *HRAS*, *KRAS* and *PIK3CA* but did not detect any mutations.

Conclusion: Pulmonary epithelial–myoepithelial carcinoma is a rare subtype of lung cancer, with only 56 previous cases reported in the English literature. The genetic alterations in pulmonary epithelial–myoepithelial carcinoma are still unclear. To our knowledge, only one study described *HRAS* mutations in pulmonary epithelial–myoepithelial carcinoma, detected in all three tumors evaluated. However, our case did not show any mutations in any of the above-examined genes.

Introduction

Epithelial–myoepithelial carcinoma (EMC) is a malignant tumor that occurs mainly in the salivary glands [1]. This tumor also arises in other locations such as the respiratory tract, minor salivary glands and lacrimal glands [1-3]. Primary salivary gland-type tumors of the lung account for 0.1–1% of all primary lung carcinomas, and most are mucoepidermoid or adenoid cystic carcinomas [4-5]. EMC is a rare subset of salivary gland-type tumor of the lung, with only 56 cases previously reported in the English literature [6]. Because of its rarity, molecular information on this tumor type is not sufficient.

Case Presentation

A 76-year-old woman was diagnosed with colon carcinoma and underwent preoperative examinations. Computed tomography coincidently revealed a regularly shaped tumor, 1.8 × 1.3 cm in size, located in the left lower lung (Figure 1). She did not have any respiratory symptoms, such as cough or dyspnea. Endoscopic submucosal dissection of the colon carcinoma was performed. Laboratory data revealed no significantly abnormal findings. Bronchoscopy revealed an endobronchial mass, and transbronchial biopsy was performed. She was diagnosed with an adenocarcinoma, and thoracoscopic left lower lobectomy with hilar and mediastinal lymph node dissection was performed.

Pathological findings

Macroscopic findings

A specimen containing the tumor was obtained at surgery. Glossy, the tumor size was 2.7 × 1.9 × 1.8 cm, and the cut surface of the tumor was whitish–yellow to gray, shiny and well-defined (Figure 2-a).

Histopathological and immunohistochemical findings

The tumor consisted of duct-like structures and polygonal-to-spindle cell features (Figure 2-b). The duct-like structures were composed of two distinct cell layers. The inner layer comprised cuboidal cells with eosinophilic cytoplasm and round nuclei, and the outer layer comprised cells with clear cytoplasm and oval nuclei (Figure 2-c). The duct-like structures contained eosinophilic material in the luminal spaces. The polygonal and spindle cells were similar to the outer-layer cells (Figure 2-d). There were no findings of necrosis or hemorrhage. Mitosis was found only in the polygonal and spindle cells (2 mitoses/10 high-power fields). Immunohistochemically, the inner-layer cuboidal cells were positive for pan-cytokeratin (Figure 3-a) and negative for vimentin, S-100, p63 (Figure 3-b) and TTF-1, suggesting an epithelial phenotype. On the other hand, the outer-layer polygonal and spindle cells were positive for S-100, p63 (Figure 3-b), HHF35 and weakly positive for pan-cytokeratin (Figure 3-a), suggesting a myoepithelial phenotype. Overexpression of p53 protein was not found.

Mutation analysis

We conducted polymerase chain reaction (PCR) followed by Sanger sequencing and pyrosequencing to investigate the mutation status of the oncogenes associated with EMC of the salivary gland [7]. Briefly, DNA from formalin-fixed paraffin-embedded tissues was extracted using TaKaRa DEXPAT (Takara Bio Inc., Shiga, Japan). The tumor component of the slides was microdissected to increase the tumor cell proportion. The PCR products were purified using the NucleoSpin Gel and PCR Clean-up, Mini kit (Marcherey-Nagel, Duren, Germany). Each purified product was directly sequenced using a forward primer and the BigDye Terminator version 3.1 cycle sequencing kit on the ABI 3730 instrument (Applied Biosystems Inc., Foster City, CA). Mutation analyses of *AKT1* (exon 2), *CTNNB1* (exon 3), *HRAS* (exons 2 and 3) and *PIK3CA* (exons 9 and 20) were performed based on the method described by Urano et al. [7]. The primer sequences are listed in Table 1. In addition, mutation analyses of *BRAF* (exon 15) and *KRAS* (exons 2 and 3) were performed using the BRAF Pyro Kit and KRAS Pyro Kit (Qiagen, Venlo, Netherlands), respectively, in real-time using pyrosequencing technology on the PyroMark Q24 System (Qiagen). As a result, no mutation in any of the six genes was detected.

Discussion

EMC is a rare malignant salivary gland tumor that accounts for <1% of all salivary gland epithelial neoplasms and nearly 2% of malignant salivary gland tumors [1, 8]. EMC of the salivary gland was first described by Donath et al. in 1972 [9]. EMC is characterized by a biphasic morphology, with an inner layer of duct-like structures composed of epithelial cells and a surrounding layer of myoepithelial cells immunoreactive for S-100 and smooth muscle actin [1,2,6]. The tracheobronchial glands are considered counterparts of the minor salivary glands in the respiratory tract and can develop similar tumors. Within this type of neoplasia, EMC of the respiratory tract is very rare, and the diagnosis is often difficult [10, 11].

Salivary gland-type tumors of the lung account for 0.1–1% of all primary lung carcinomas, among which mucoepidermoid carcinoma is the most frequently observed histological subtype, followed by adenoid cystic carcinoma and then EMC [4,5]. Our case was diagnosed as adenocarcinoma at the time of biopsy. Recently, Nakashima et al. conducted a literature review of 56 patients (32 females and 24 males; average age [range], 56 [7–81] years) with pulmonary EMC [6]. Of these, 45 patients had tumors localized in the central airway within segmental bronchi appearing to be endobronchial masses. The size of the tumors varied from 0.7 to 16 cm in diameter (average 2.5 cm). According to the histopathological characteristics, three distinct histological subtypes of pulmonary EMC were reported: (1) EMC with two ductal components, defined as a characteristic feature of this tumor, (2) EMC with a solid component consisting mainly of spindle- and polygonal-shaped myoepithelial cells, and (3) EMC consisting mainly of myoepithelial cells with increased nuclear atypia, referred to as myoepithelial anaplasia [6, 12-14]. Seethala et al. reported that patients with myoepithelial anaplasia had a poorer survival compared with others [15]. Our case is of typical EMC featuring two ductal components and immunohistochemically.

The genetic alterations associated with pulmonary EMC are still uncertain because of the rarity of this tumor, with only one report so far, to our knowledge [7]. On the other hand, several genetic mutations have been detected in EMC of the salivary glands [7,16,17]; *HRAS* mutations are the most frequently detected, followed by *PIK3CA* and *AKT1* mutations. Urano et al. described that all three pulmonary EMCs that they evaluated contained *HRAS* mutations [7]. However, our case did not show any mutations in *AKT1*, *HRAS* or *PIK3CA*. Although the number of reported cases is very small, the frequency of *HRAS* mutations in pulmonary EMC is 75% (3/4 cases). At this time, we cannot conclude whether the genetic alterations in pulmonary EMC are similar to those of other EMC types. Typical cases of pulmonary EMC are easy to diagnose, whereas atypical cases can be difficult to distinguish. Determination of the characteristic gene mutations will be useful for differentiating pulmonary EMC from other salivary gland tumors of the lung.

In conclusion, we report a case of pulmonary EMC containing no *AKT1*, *HRAS*, or *PIK3CA* mutations. Further examinations will be needed.

Abbreviations

EMC: epithelial–myoepithelial carcinoma; PCR: polymerase chain reaction

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

NY and TS: conception and writing of manuscript. NY and HS: collection of clinical data. NY, AS, KN, MS, RS, MO and NU: pathological diagnosis and immunohistochemical analyses. NY: collection of the samples for the molecular analyses. NY, HS and TS: revision of the manuscript. All authors read and approved the final manuscript prior to submission.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent was obtained from the patient for the publication of this case report.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1 PCR primers used for Sanger sequencing

Gene	Direction	Sequence (5’ to 3’)
AKT1 exon 2	Forward	AGGCACATCTGTCCTGGCAC
	Reverse	AAATCTGAATCCCGAGAGGCC
CTNNB1 exon 3	Forward	TTTGATGGAGTTGGACATGG
	Reverse	AAAATCCCTGTTCCCACTCA
HRAS exon 2	Forward	CAGGCCCCTGAGGAGCGATG
	Reverse	TTCGTCCACAAAATGGTTCT
HRAS exon 3	Forward	TCCTGCAGGATTCCTACCGG
	Reverse	GGTTCACCTGTACTGGTGGGA
PIK3CA exon 9	Forward	TGACAAAGAAGAGCTCAAAGC
	Reverse	TTAGCACTTACCTGTGACTCCA
PIK3CA exon 20	Forward	TGATGACATTGCATACATTCG
	Reverse	TGTGTGGAAGATCCAATCCA

Figures

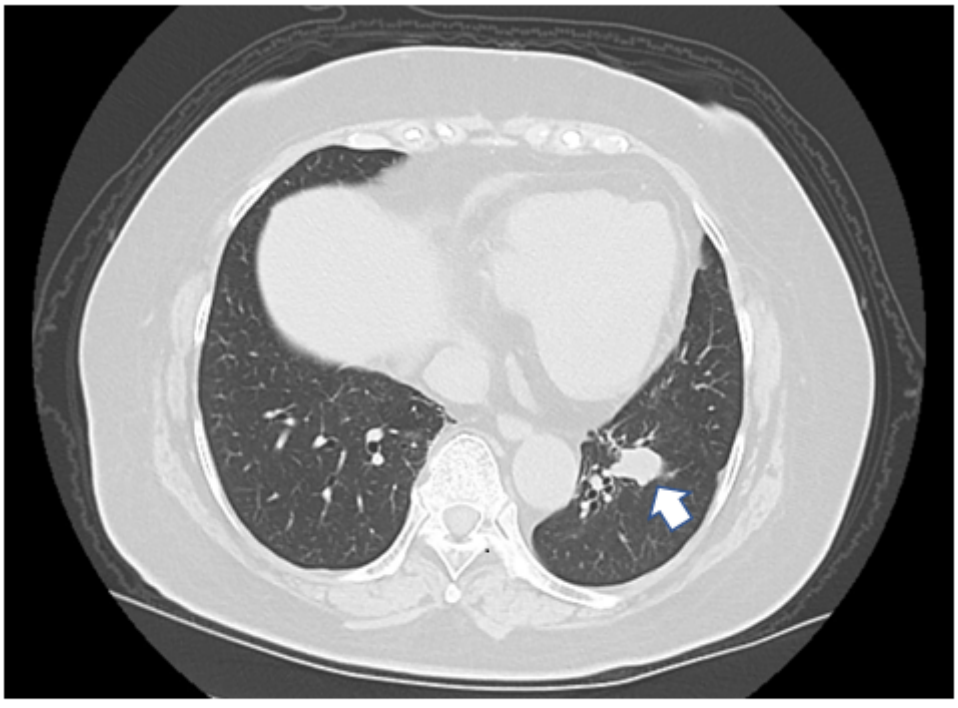

Figure 1

Figure 1

Computed tomography revealed a regularly shaped tumor, 1.8 × 1.3 cm in size, located in the left lower lung (white arrow).

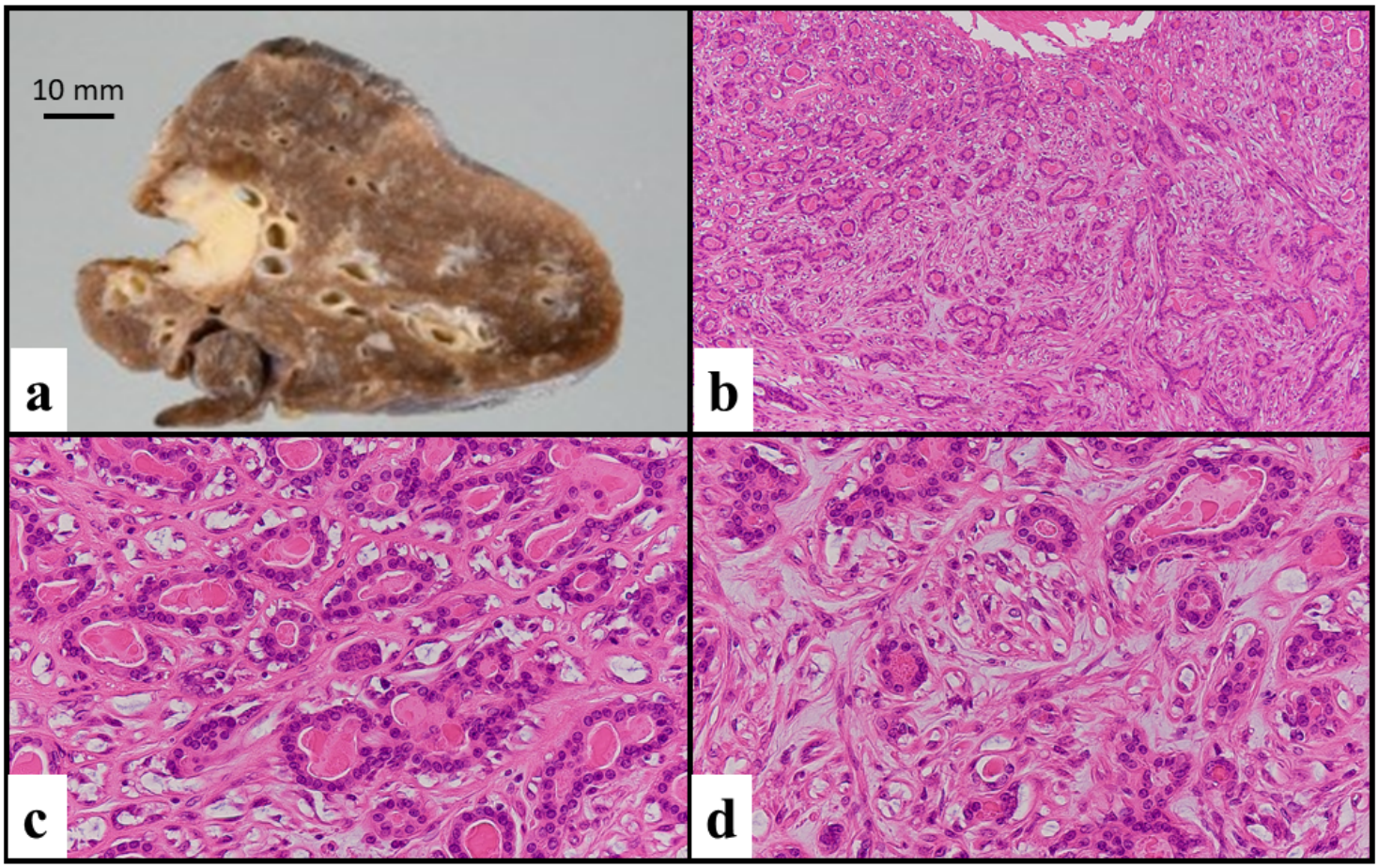

Figure 2

Figure 2

Macroscopic and microscopic findings. (a) Cut surface of the surgical specimen. The tumor size was 2.7 × 1.9 × 1.8 cm, whitish–yellow to gray, shiny and well-defined. (b) The tumor consisted of duct-like structures and polygonal-to-spindle-cell features. (c) The duct-like structures comprised two distinct cell layers. The inner cell layer was composed of cuboidal cells with eosinophilic cytoplasm and round nuclei, and the outer cell layer was composed of cells with clear cytoplasm and oval-to-fusiform nuclei. (d) The polygonal and spindle cells were similar to the outer-layer cells and had clear cytoplasm.

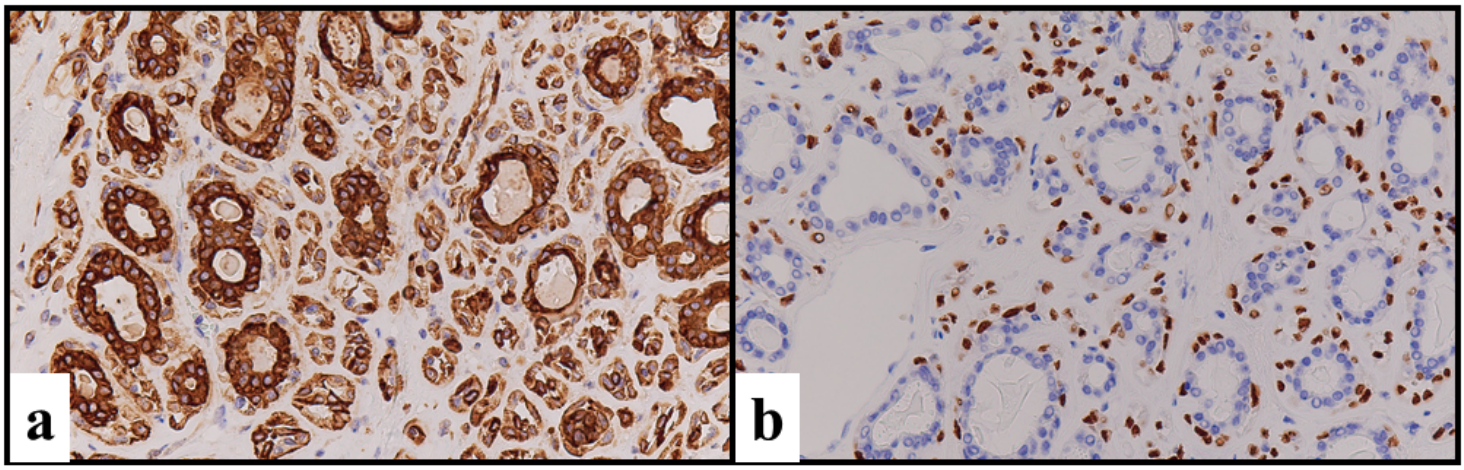

Figure 3

Figure 3

Immunohistochemical findings. (a) The cuboidal cells in the inner layer were positive for pan-cytokeratin, and the outer layer of cells and polygonal/spindle cells were weakly positive for pan-cytokeratin. (b) The cuboidal cells in the inner layer were negative for p63, whereas the outer layer of cells and polygonal/spindle cells were positive for p63.