Case Report

DOG-1 positive primary acinic cell carcinoma of the lung and investigation of molecular status

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Summary
Primary acinic cell carcinoma (ACC) of the lung is an extremely rare neoplasm that more often arises near to a right bronchus. It is characterized by two populations of clear and dark eosinophilic cells, arranged in a glandular acinar pattern. Mitosis are rare and tumor cells show small and eccentric nuclei. Positive stain for PAS, PAS-D, cytokeratin, A1AT and A1ACT is reported, while TTF1, p40, synaptophysin, SMA, and S100 are substantially negative. DOG-1 positive stain was observed in ACC of the salivary glands and its negativity was proposed to distinguish between primary and metastatic ACC of the lung. Here, we report the 30th case of primary ACC of the lung, describing the immunohistochemical positivity for DOG-1 and the molecular status of the neoplasm for the first time.

Key words: lung cancer, salivary gland-type tumor, acinic cell carcinoma, NR4A3, DOG-1

Introduction

Acinic cell carcinoma (ACCs) is an uncommon epithelial neoplasm of the salivary glands that most often affects the parotid gland. Primary ACC has also been described in other anatomical sites including the breast, nasal cavity, and lung. Primary ACC of the lung is an extremely rare neoplasm that is histologically similar to its salivary counterpart. This entity was described for the first time by Fechner et al. in 1972;1 since then, only 29 cases have been reported in the English literature. DOG-1 positive stain was described in ACC of the salivary glands and its negativity was proposed to distinguish between primary and metastatic ACC of the lung.2,3 Here, we report a new case of primary ACC of the lung, evaluating the utility of DOG-1 in diagnosis and describing the molecular status of the neoplasm.

Case presentation

A 53-year-old Hispanic ex-smoker man was admitted to the emergency room of Campus Bio-Medico of Rome (CBM) for hemoptysis in December 2021. Computed Tomography (CT) and Positron Emission Tomography - Computed Tomography (PET-TC) revealed a solid mass adjecting within the main right bronchus (MRB) with a low uptake (SUV max:
2.28) (Fig. 1A). Tissue samples from Endobronchial Ultrasound-guided Transbronchial Needle Aspiration (EBUS-TBNA) and bronchial alveolar lavage (BAL) were collected in UCBM Pathology Dept. After the cancer diagnosis, a right superior lobectomy with lymphadenectomy of stations II, IV, VII, VIII, IX, X and XI was performed.

**Histopathological, immunohistochemical and molecular evaluation**

The biopsy sample was formalin-fixed and paraffin-embedded (FFPE) while the cytological sample was processed with the cell block technique. 5 μm sections and hematoxylin-eosin (HE) stain were cut. On microscopical examination, a neoplasm with a glandular growth pattern composed by eosinophilic cells and eccentric nuclei without nucleoli was observed; immunohistochemical analysis revealed strong positivity for cytokeratin (AE1AE3 PCK26, ready to use, Roche®) and negativity for TTF1 (8G7G31, ready to use, Roche®), p40 (BC28, ready to use, Roche®) and synaptophysin (SP11, ready to use Roche®). Immunostaining was performed by BenchMark ULTRA system - Ventana.

Evaluation of the of surgical specimen revealed the presence of a polypoid endobronchial neoplasm of the main right bronchus (MRB) with a maximum dimension of 2.2 cm, located at the distance of cm 1 from the bronchial margin of surgical resection. Visceral pleura, lung margin of surgical resection, and lung parenchyma were macroscopically far from the tumor. A frozen section of the neoplasm was set up for the intraoperative histological examination that confirmed the diagnosis of a glandular carcinoma with low-grade atypia. Because of the cribriform aspects of the tumor and its relationship with the peri-bronchial glands, a more specific diagnosis was postponed until definitive diagnostic evaluation, with the need to exclude a salivary-type carcinoma.

Histological evaluation of the resection specimen showed tumor glands with eosinophilic intra-glandular secretion. The tumor was also present close to the

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**Figure 1.** (A) PET-TC showing the endobronchial mass within the main right bronchus (MRB) (red arrow) with a low uptake (SUV max 2.28). (B) Tissue sample from the surgical specimen, H&E, 40x. The monomorphic cells are characterized by eosinophilic cytoplasm, eccentric nuclei, and the acinar pattern. Note the eosinophilic secretion within the lumen of the acini. (C) A red line divides the clear cells (on the right) and dark cells (on the left), H&E, 20x. (D) The image shows the tumor cells next to peri bronchial glands (green arrow: tumor cells, yellow arrow: normal peri bronchial cells).
normal peri bronchial glands, and it was characterized by both clear and dark cells; no mitosis, necrosis or psammomatous bodies were found (Figs. 1B-D). No lymph node metastases were present. Further histological and immunohistochemical analysis performed on the FFPE tissue slides from the surgical specimen revealed extra and intracellular positivity for PAS-D (Periodic-Acid Schiff with diastase), cytoplasmatic positivity for alpha-1 anti-trypsin (A1AT) (polyclonal, ready to use, Dako Corporation®), nuclear SOX10 positivity (SP267 clone, ready to use, Roche®) and apical membranous positivity for DOG-1 (SP31, ready to use, Roche®); TTF1, p40, synaptophysin, smooth-muscle actin (SMA) (1A4, ready to use, Roche®) and S100 (4C4.9, ready to use, Roche®) were negative (Figs. 2, 3). Surprisingly, our case showed luminal positivity for DOG-1, which was proposed to support the diagnosis of metastatic ACC from salivary glands. The typical histological appearance together with the absence of a basal cell layer evaluated by p40 and S100 allowed us to exclude the diagnosis of adenoid cystic carcinoma or epithelial-myoepithelial carcinoma. Since NR4A3 rearrangement status was reported in ACC, we performed FISH analysis to complete tumor characterization (ZytoLight SPEC NR4A3 dual-color break-apart probe; Z-2145-50; ZytoVision) with a negative result.

The evaluation of molecular status with FISH (fluorescence in situ hybridization) for ALK and ROS-1 translocation and MET amplification and with DNA-NGS (next generation sequencing) for ALK, BRAF, EGFR, ERBB2, FGFR3, HRAS, IDH1, IDH2, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA, RET and ROS1 did not reveal re-arrangement or mutations in any gene analyzed.

**Clinical evaluation and follow-up**

Considering the diagnosis of a salivary type tumor and the positivity for DOG-1, a head and neck CT scan and a parotid ultrasound were performed. No evidence of salivary gland tumor was detected, supporting the diagnosis of primary ACC of the lung. The presence of granulation tissue on the bronchial anastomosis was reported at the control bronchoscopy.

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**Figure 2.** (A) Cytokeratin (CKAE1-AE3) positive stain, 20x. (B) A1AT positive stain, 40x; the cytoplasm of tumor cells shows a granular positivity. (C) DOG1 positive luminal stain, 40x. Similarly, from primary ACC of salivary glands, our case shows a positive stain for DOG1. Note the apical membranous positivity. Neoplasia lacks complete membranous and cytoplasmatic positivity; this pattern resembles that of normal salivary tissue. (D) PAS-D positive stain, 40x. Note the positivity in luminal secretions and in the cytoplasm as various size globules.
performed one month after the lobectomy; a biopsy on this site revealed the presence of squamous metaplasia of the bronchial epithelium. The first radiological follow-up was planned at three months after the lobectomy; a CT will be planned once every six months.

**Discussion**

Salivary gland-type tumors of the lung include primary lung tumors resembling primary those of salivary glands. The 2021 WHO includes in this group pleomorphic adenoma of the lung, adenoid cystic carcinoma of the lung, epithelial-myoepithelial carcinoma of the lung, mucoepidermoid carcinoma of the lung, hyalinizing clear cell carcinoma of the lung, myoepithelioma and myoepithelial carcinoma of the lung. Primary ACC of the lung is an extremely rare and quite indolent salivary gland-type tumor of the lung, not discussed in the 2021 WHO. Only 29 cases were previously described in the English literature (Fig. 4). There is not a clear gender predilection and both adults and children could be potentially affected, with the mean age at the diagnosis of 43 years old. Two pediatric patients had a history of bronchial foreign body. More often the tumor was found close to a right bronchus and the diagnosis was performed after respiratory or

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**Figure 3.** (A) P40 negative stain, 40x; note the positive control in the bronchial basal layer (black arrow). (B) TTF1 negative stain, 20x. (C) SOX10 positive stain, 40x. Note the nuclear positive stain in tumor cell and the negativity in the respiratory epithelium. (D) NR4A3 FISH with ZtoLight SPEC NR4A3 Dual Color Break Apart Probe (Z-2145-50). The exam did not reveal NR4A3 rearrangement. Note the two green/orange fusion signals per cell.

**Figure 4.** Geographical distribution of all described cases of acinic cell carcinoma in the English literature. Orange: America; Green: Europe; Brown: Africa; Yellow: Asia; Purple: Australia. Graphics program: InkScape®.
obstructive symptoms; less frequently, the lesion was discovered as incidental finding after a routine chest X-ray. Two cases were reported with lymph nodes metastasis and only one of them showed a recurrence of the neoplasm. Table I summarizes all clinical features of the 30 reported cases.

Histologically, diagnosis of primary acinic cell carcinoma of the lung is particularly challenging because it is mainly based upon morphological features. Fechner et al. described for the first time two populations of cells in primary acinic cell carcinoma of the lung: dark cells resembling the normal serous cells of the bronchial submucosal glands and a majority component of light cells, similar to the acinic cell tumor

Table I. Clinical and anatomical data of the 30 cases of acinic cell carcinoma.

| Author             | n° of cases | Country         | Sex | Age at diagnosis | Clinical presentation                        | Anatomical site|Location   | Size of tumor (cm) |
|--------------------|-------------|-----------------|-----|------------------|---------------------------------------------|---------------|-----------|-------------------|
| Fechner et al.     | 1           | America (USA)   | M   | 63               | Incidental                                  | RLL\parenchymal|           | 4,2               |
| Katz et al.        | 1           | Europe (Israel) | F   | 12               | Recurrent pulmonary infections              | RML (Bronchus intermedia) | 0,8       |
| Heard et al.       | 1           | Europe (England)| M   | 54               | Three-year history of hemoptysis            | Trachea       |           | 2,2               |
| Gharpure et al.    | 1           | Asia (India)    | M   | 36               | Persistent Cough, hemoptysis and weakening of voice | RML\endobronchial | 4         |
| Moran et al.       | 1           | America (USA)   | F   | 44               | Incidental                                  | RML\subpleural |           | 3,5               |
|                    | 2           |                 | F   | 48               | Incidental                                  | LUL\parenchymal |           | 4                |
|                    | 3           |                 | F   | 50               | Incidental                                  | RML\subpleural |           | 1,7               |
|                    | 4           |                 | M   | 63               | Persistent cough                             | RML\endobronchial |           | 1,2               |
|                    | 5           |                 | F   | 75               | Incidental                                  | RUL\subpleural |           | 1,5               |
| Horowitz et al.    | 1           | Europe (Israel) | M   | 31               | Persistent cough                            | Trachea       |           | 2                |
| Anisari et al.     | 1           | America (USA)   | M   | 59               | Persistent cough, hemoptysis                | Trachea       |           | 3                |
| Ukoha et al.       | 1           | America (USA)   | M   | 64               | Incidental                                  | LUL B-6\endobronchial |           | 3                |
| Lee et al.         | 1           | Asia (Malesia)  | F   | 30               | Incidental                                  | RLL\subpleural |           | 2                |
| Watanabe et al.    | 1           | Asia (Japan)    | M   | 58               | Persistent cough                            | RML\endobronchial |           | 1,5               |
| Sabaratnam et al.  | 1           | Africa (South Africa) | M   | 4               | Hemothysis and night sweats                  | LUL\endobronchial |           | 3                |
| Rodriguez et al.   | 1           | Europe (Italy)  | M   | 70               | Incidental                                  | RLL\intraparenchymal |           | 4                |
| Tsukayama et al.   | 1           | Asia (Japan)    | M   | 54               | Hoarseness                                  | Trachea       |           | 5                |
| Vongsivavilas et al.| 1           | Asia (Thailand)| F   | 37               | Incidental                                  | RUL\NF        |           | 3,3               |
| Gamal et al.       | 1           | Asia (Japan)    | F   | 47               | Incidental                                  | LLL B-9\endobronchial |           | 1,8               |
| Cho et al.         | 1           | Asia (Korea)    | M   | 68               | Incidental                                  | LLL\intraparenchymal |           | 1,8               |
| Sano et al.        | 1           | Asia (Japan)    | F   | 55               | Persistent cough                            | RUL S-3       |           | 1,3               |
| Zhang et al.       | 1           | Asia (China)    | M   | 31               | Persistent cough                            | RUL\subpleural |           | 4,5               |
| Nie et al.         | 1           | Asia (China)    | F   | 10               | Persistent cough, hemoptysis                | LUL\U         |           | 1,5               |
|                    | 2           |                 | M   | 25               | Persistent cough, chest pain                | RUL\U         |           | 2,2               |
|                    | 3           |                 | M   | 37               | Hemothysis                                  | RLL\U         |           | 1                |
|                    | 4           |                 | F   | 8                | Persistent cough, hemoptysis                | LML\U         |           | 0,4               |
|                    | 5           |                 | M   | 28               | Persistent cough, hemoptysis                | RML\U         |           | 1                |
|                    | 6           |                 | F   | 53               | Hemothysis                                  | RML\U         |           | 1,5               |
| Chen et al.        | 1           | Asia (China)    | F   | 27               | Persistent cough, hemoptysis                | RUL\endobronchial |           | 8,6               |
| Our case           | 1           | Italy (Europe)  | M   | 53               | Persistent cough, hemoptysis                | RUL\endobronchial |           | 2,2               |

Abbreviations: RLL: right lower lobe; RML: right middle lobe; RUL: right upper lobe; LLL: left lower lobe; LUL: left upper lobe.
of the parotid\cite{1}. Some authors signaled the presence of psammomatous bodies within the tumor. PAS and PAS-D histochemical stains were positive in all cases when performed. Immunohistochemistry positivity for Alpha 1-antichymotrypsin (A1ACT) (8/10) and A1AT (3/3) were also described. Nie et al. proposed for the first time the use of DOG-1 in immunohistochemistry to distinguish between primary acinic cell carcinoma of the lung and metastatic ACC from salivary glands\cite{2}. DOG-1 was initially adopted to confirm the diagnosis of gastrointestinal stromal tumor (GIST); subsequently, its expression in luminal plasmalemma of salivary tissue was described. Similarly, DOG-1 staining in salivary ACC shows intense apical membranous positivity around lumina as well as complete membranous and variable cytoplasmatic positivity\cite{4}. In seven cases of primary ACC of the lung, in which DOG-1 was evaluated, no positivity was detected\cite{2,3}. Surprisingly, our case was the first one characterized by DOG-1 positive stain; this finding suggests considering DOG-1 positivity with caution to distinguish between primary from metastatic ACC. The SOX10 also supports our diagnosis since reported as positive in salivary glands and salivary type ACC. Moreover, in salivary glands ACC and sinonasal ACC, the t(4;9) NR4A3 translocation was described while, similarly to the breast ACC, the rearrangement was negative in our case\cite{5}. To the best of our knowledge, NR4A3 rearrangement status was not previously investigated in lung ACC.

In conclusion, we report the unique case of primary ACC of the lung with a DOG-1 luminal expression. Furthermore, the molecular status of this tumor was investigated for the first time and, differently from salivary glands and sinonasal ACC, our findings suggest no diagnostic role for NR4A3 translocation in confirming the diagnosis of lung ACC. However, the lack of NR4A3 rearrangement could be useful to distinguish ACC of the lung and breast from salivary gland and sinonasal ACC. ACC of the lung is not still mentioned within the WHO section of Salivary Type tumor of the lung which could contribute to underestimate the prevalence of this indolent neoplasm. Ignoring this tumor can lead to a misdiagnosis of lung adenocarcinoma with a consequent overtreatment. Sharing all these cases is crucial to raise awareness of this entity and reduce misdiagnosis.

**Conflicts of interest**
None of the authors has a conflict of interest.

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**Ethical consideration**
The information contained in this manuscript complies with the journal's ethical standards.

**Author contributions**
LN: reviewed the literature and wrote the manuscript; GP contributed to the histological diagnosis, evaluated the molecular analysis and reviewed the manuscript; PG made the histological diagnosis and reviewed the manuscript; ST provided contribution for immunohistochemical data and reviewed the manuscript; GS and DR performed the molecular analysis; LF and PC provided clinical and radiological data. All authors gave final approval for publication.

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