71186_Auto_Edited-check.docx

WORD COUNT	3264	TIME SUBMITTED	15-FEB-2022 09:25PM
		PAPER ID	82599474

Name of Journal: *World Journal of Clinical Cases*

Manuscript NO: 71186

Manuscript Type: CASE REPORT

Bronchiolar adenoma with unusual presentation: Two case reports

Du Y *et al*. Two cases of distal BA

Abstract

BACKGROUND

The clinicopathological features, immunohistochemical characteristics and genetic mutation profile of two unusual cases of distal bronchiolar adenoma were retrospectively analyzed and the relevant literature were reviewed.

CASE SUMMARY

Case 1, female, 63 years old. She had a mixed ground-glass nodule, with mild cells in morphology, visible cilia, and bilayer structures in the focal area. Immunohistochemistry for P63 and cytokeratin (CK)5/6 revealed the lack of a continuous bilayer structure in most areas, and no mutations were found in epidermal growth factor receptor, anaplastic lymphoma kinase, ROS1, Kirsten rat sarcoma (KRAS), PIK3CA, BRAF, Human epidermal growth factor receptor-2 (HER2), REarranged during Transfection, and neuroblastoma RAS genes. Case 2, female, 58 years old. She presented with a solid nodule; most cells were observed to be medium sized, the nuclear chromatin was pale and homogeneous, local cells had atypia, and cilium was found locally. Immunohistochemistry for P63 and CK5/6 showed mild cell morphology without expression, whereas the heteromorphic cells showed a bilayer structure. The same nine genes were analyzed, and positive HER2 gene mutation was identified.

CONCLUSION

Some unresolved questions remain to be answered to determine whether the lesion is a benign adenoma or a part of the process of malignant transformation from benign adenoma of the bronchial epithelium. Furthermore, whether lesions with atypical bilayer structures are similar to atypical hyperplastic lesions of the breast remains to be elucidated. Moreover, some clarity on whether these lesions can be called atypical bronchiolar adenoma and whether they are invasive precursor lesions is needed. Future

studies should examine the diagnostic significance of HER2 gene mutation and its value as a prognostic indicator.

Key Words: Bronchiolar adenoma; Human epidermal growth factor receptor-2 gene; Thoracic tumors; Cellular atypia; Ciliated muconodular papillary tumor; Case reports

Du Y, Wang ZY, Zheng Z, Li YX, Wang XY, Du R. Bronchiolar adenoma with unusual presentation: Two case reports. *World J Clin Cases* 2022; In press

Core Tip: In terms of morphology, case 1 had mild cells, visible cilia, and bilayer structures in the focal area. Immunohistochemistry for P63 and cytokeratin (CK)5/6 revealed the lack of a continuous bilayer structure in most areas, and no mutations were found in the genes. In case 2, most cells were medium-sized. Furthermore, local cells had atypia, and cilium was found locally. Immunohistochemistry for P63 and CK5/6 revealed that only heteromorphic cell regions showed a bilayer structure. A positive Human epidermal growth factor receptor-2 gene mutation was identified. Further research is needed to know whether these lesions can be called atypical bronchiolar adenoma and whether they are invasive precursor lesions.

INTRODUCTION

Bronchiolar adenoma (BA) clinically presents as a benign or potentially malignant tumor. BA is thought to originate from the bronchiolar epithelium, which has a series of cell differentiation in a bilayer arrangement of multipartite epithelial cells and basal cells. BA is expected to gain more widespread recognition in the 2021 edition of the World Health Organization classification of thoracic tumors[1,2]. The histological variants of BA can be distinguished as the "classic" ciliated muconodular papillary tumor (CMPT) (proximal type) and "non-classic" CMPT (distal type). The histological features of CMPT include a bilayer structure composed of a continuous basal cell layer and luminal cell layer (comprising varying proportions of mucinous cells, ciliated cells,

Clara cells, and/or type II alveolar epithelial cells)[3,4]. BA often exhibits only focal or no papillary architecture and contains variable numbers of ciliated and mucinous cells, with some lesions entirely lacking one or both of these components[3,4]. A recent study revealed the involvement of potential gene mutations that may be responsible for the neoplastic nature of BA[5]. Mutations in the anaplastic lymphoma kinase (ALK), Kirsten rat sarcoma (KRAS), BRAF, AKT1, and epidermal growth factor receptor (EGFR) genes were identified in BA, and these genes were considered as susceptible driver oncogenes that eventually lead to the development of neoplasms[6-9]. Meanwhile, in a recent study on BA, Chang *et al*[2] identified BRAF V600E mutations (38%), EGFR exon 19 deletions (10%), EGFR exon 20 insertions (10%), KRAS mutations (24%), and HRAS mutations (5%), thus supporting a truly neoplastic process of BA.

Cases of a single- or double-layer bronchial adenoma with atypical bronchiolitis are rare. Here, we report two cases with BA confirmed by imaging, morphology examination, immunohistochemical characteristics, and genetic tests.

CASE PRESENTATION

Chief complaints

Case 1: On examination at the local hospital in September 2020, a 63-year-old female patient was found to have pulmonary nodules.

Case 2: A 58-year-old female patient underwent chest computed tomography (CT) examination in our hospital January 19, 2021 and was identified as having nodules in the right upper lobe of the lung.

History of present illness

Case 1: Upon examination at the local hospital in September 2020, she was found to have pulmonary nodules; she did not report having cough or expectoration, chest pain, chest tightness, or other symptoms. No further specific diagnosis was made or treatment advised. Since the discovery of the nodule, the patient has been lucid and

mentally healthy with normal diet and sleep. The laboratory reports for urine and stool were normal, and there were no significant changes in weight.

Case 2: She underwent chest CT examination in our hospital on January 19, 2021 and was identified as having nodules in the right upper lobe of the lung. Except for occasional cough and expectorate phlegm, she showed no other signs and symptoms similar to those observed in case 1.

History of past illness

The patients had a free previous medical history.

Personal and family history

The patients had no personal and family history.

Physical examination

Case 1: After admission to the hospital, the patient's temperature was 36.6 ℃, heart rate was 58 bpm, respiratory rate was 16 breaths per minute, and blood pressure was 112/59 mmHg.

Case 2: The patient's temperature was 36.9 ℃, heart rate was 67 bpm, respiratory rate was 16 breaths per minute, and blood pressure was 120/67 mmHg.

In both cases, chest examination found that the trachea was in the center, the thorax was not deformed, the breath sounds of the lungs were slightly thicker, and no obvious dry and wet rales were heard.

Laboratory examinations

Case 1: The biochemical indicators showed the following results: Cancer embryonic antigen (CEA) was 0.71 ng/mL (reference range: 0-5 ng/mL), neuron-specific enolase (NSE) was 11.94 ng/mL (reference range: 0-35 ng/mL), cytokeratin protein (CK19) was

2.33 ng/mL (reference range: 0-3.3 ng/mL), squamous cell carcinoma antigen (SCC) was 0.8 ng/mL (reference range: ≤ 1.5 ng/mL), carbohydrate antigen 125 (CA125) was 6.7 U/mL (reference range: 0-35 ng/mL), and pro-gastrin-releasing peptide (pro-GRP) was 24.99 pg/mL (reference range: ≤ 63 pg/mL).

Case 2: The biochemical indicators showed the following results: CEA was 3.22 ng/mL, CA125 was 9 U/mL, NSE was 11.21 ng/mL, CK19 was 1.87 ng/mL, SCC was 0.7 ng/mL, and pro-GRP was 27.65 pg/mL, all of which were normal.

Imaging examinations

Case 1: On December 7, 2020, the findings of thoracic enhanced CT performed in our hospital revealed bronchitis, right lower pulmonary bullae, and subpleural nodules and pleural traction in the lower lobe of the right lung of the patient (Figure 1A).

Case 2: She underwent chest CT examination in our hospital January 19, 2021 and was identified as having nodules in the right upper lobe of the lung (Figure 1B).

Case data: Surgical specimens were fixed with 4% neutral buffer formaldehyde solution (18-24 h) and embedded with paraffin; sections (4 μm thick) were subjected to hematoxylin-eosin staining[10], and immunohistochemistry analyses.

Immunohistochemical staining: Immunohistochemical analyses were performed on paraffin-embedded sections using primary antibodies against the following proteins: P40, P63, P53, thyroid transcription factor 1, CK5/6, CD34, Ki-67, and collagen IV. All primary antibodies were purchased from Fuzhou Maixin Biotechnology Co., Ltd. (Fuzhou, China). Immunohistochemistry was performed according to the manufacturer's instructions. Polybutylene succinate was used as a negative control. Staining was performed using the Roche Benchmark XT medical system, shanghai.

Genetic testing: Mutations in the EGFR, ALK, ROS1, KRAS, PIK3CA, BRAF, Human epidermal growth factor receptor-2 (HER2), REarranged during Transfection, and neuroblastoma RAS genes were detected using the ADX Arms and the Amoydx FFPE DNA/RNA Tissue Kit (Xiamen Ade Biomedical Technology Co., Ltd.). All experimental procedures were performed strictly according to the manufacturer's instructions.

Surgical findings

Case report 1: A small subpleural nodule was found in the lower lobe of the right lung. The nodule was approximately 0.6 cm in diameter and did not involve the visceral pleura. A wedge-shaped resection of the nodule was performed.

Case report 2: After performing preoperative puncture and locating the right upper lobe nodules, a solid nodule with a diameter of 0.7 cm was palpated around the lobe. The nodule of the right upper lobe was excised by a wedge-shaped incision.

Pathological examination

Gross pathological examination: Case report 1: A piece of grayish red lung tissue was removed by wedge resection; the tissue measured 9 cm × 3.5 cm × 2 cm. The pleura was grayish red and smooth; a grayish white nodule was found by multi-section incision. The nodule measured 0.6 cm × 0.5 cm × 0.3 cm. The texture of the nodule was similar to that of normal salivary glands. It showed a clear boundary attached to the surrounding normal lung tissue, which was 2 cm away from the anastomosis line, and the remaining section was grayish red and soft.

Case report 2: Upon gross pathological examination, we identified a piece of grayish red lung tissue measuring 10 cm × 4 cm × 2 cm. A partial incision was made by the surgeon. The pleura was grayish red and smooth; a grayish white nodule was later found upon incision. The nodule measured 0.6 cm × 0.5 cm × 0.5 cm. The texture of the nodule was similar to that of normal salivary glands. The nodule showed clear

boundaries and was attached to the pleura 2 cm away from the anastomosis line. The remaining section was grayish red and soft.

Microscopic pathological examination and immunohistochemistry findings

Case report 1: At low magnification (100 ×), the tumor boundary was relatively clear, and air cavities were present. The pulmonary lobular artery and bronchioles were observed, and the peripheral stromal lymphocytes were localized (Figures 2A, 2B and 2E). At high magnification (200 × and 400 ×), most tumor cells were arranged in a monolayer structure, and the local part appeared as a bilayer structure. Morphologically, the cells were observed to be of medium size (the size of the nucleus and normal phagocytic nuclei was equivalent in the alveolar space); the nuclear chromatin was pale and homogeneous, and local cilia were seen (Figure 2C-G). Thyroid transcription factor 1 (TTF1) was expressed in bronchioles and the peripheral alveolar epithelium, with the only difference being in the intensity of expression. The results of P40, P63, and CK5/6 staining were the same, and staining was positive only in the bilayer structure of the tumor (Figure 3).

Case report 2: At low magnification (100 ×), most cells appeared with moderate density, focal hyperplasia, and stroma within the focal lymphocytic infiltration; at medium to high magnification (200 × and 400 ×), the tumor cells appeared arranged in an acinar structure and accessory wall structure; most cells were observed to be medium sized, the nuclear chromatin was pale and homogeneous, and cilia were seen. The focal nucleus was enlarged and atypical (Figure 4). TTF-1 was positive; the results for P63 and CK5/6 staining were the same, that only basal cells were seen in the hyperplasia area. CD34 showed the presence of alveolar structure, and the Ki-67 index was low (Figure 5).

Genetic tests: Genetic tests were performed in the patient DNA sample to check for mutations in EGFR, ALK, ROS1, KRAS, PIK3CA, BRAF, HER2, RET, and NRAS genes.

Case report 1: All genes were negative for mutations.

Case report 2: Positive HER2 gene mutation was detected.

FINAL DIAGNOSIS

Based on the histological characteristics and results of immunohistochemical staining, the tumor was diagnosed as BA with unusual presentation.

TREATMENT

Complete wedge resection was performed in the Thoracic Surgery Department of Liaocheng People's Hospital.

OUTCOME AND FOLLOW-UP

After surgical resection, neither patient received radiotherapy or chemotherapy. At the time of writing this report, which is 11 and 12 mo postoperatively for the two patients, the patients have recovered well without signs of disease.

DISCUSSION

In 2018, BA was proposed by Chang *et al*[2] as a new type of lung tumor, defined as a group of pulmonary tumors that could be benign or have a potential for malignant activity depending on the epithelial cell composition of the bronchiolar anatomy. These include classic CMPT and non-classic CMPT, which differ according to histological aspects. BAs can be further divided into two categories-proximal (similar to proximal bronchioles) and distal (similar to respiratory bronchioles) types-based on the histomorphology (comparing histological features of different grades of bronchial epithelial cells and their similarity with the bronchioles) and immunohistochemical characteristics. Proximal-type BAs comprise numerous prominent mucinous cells and are well defined with ciliated cells and intact basal layer cells that are arranged in a papillary or flattened pattern. Conversely, the distal form usually shows a flattened

pattern and comprises few mucinous cells, cubic cells, and/or ciliated cells. Although there is some overlap between the characteristics of the two types, some lesions may lack one or both of these components. Zheng *et al*[4] reported that mucinous and papillary components are usually present throughout classic CMPTs but may be absent in their "non-classic" counterparts. Furthermore, Shao *et al*[3] also found mixed-type BAs with monolayered lesions[2,4,11].

In this study, two very rare cases of BAs comprising mucinous cells were reported. The cell arrangement observed showed a flattened pattern, indicating the distal type of BA. Although tumor cells formed an adenoid or papillary structure, the ciliary structure could be seen locally in lumen cells. Many studies have reported that the ciliary structure in lumen cells can distinguish this type of tumor from an adenocarcinoma, which is an important characteristic to help differentiate between the two tumor types[12]. However, in the two current cases, not every lumen cell had cilia, and the basal cells could not be easily observed, thus causing some difficulties in diagnosis, particularly when the specimen was frozen. Therefore, interpretations should be made considering both atypia of cells and their arrangement. In our two cases, most cells were loosely arranged, the morphology of glandular epithelial cells was mild, and the cytoplasm of local cells was transparent. Few intranuclear inclusion bodies were seen under a high-power microscope; this combined with a marginally increased nucleoplasmic ratio suggested that the lesion was benign.

In the second case, atypic cells and the absence of the entire lesion's bilayer structure complicated the diagnosis. However, these lesions were different from adenocarcinoma *in situ* Automatic Identification System (AIS) and invasive adenocarcinoma. The tumor cells of AIS comprise type II alveolar epithelial cells and/or Clara cells, which grow along the original alveolar wall without destroying the alveolar structure. In this case, ciliated columnar cells or mucinous cells were rarely present, and cell atypia was more pronounced than in BA. The boundary of invasive adenocarcinoma is not discernible; the alveolar structure is destroyed and the growth is rapid. In addition, the

micropapillary structure can be seen in the lumen and necrosis is visible; cell atypia is evident, and nuclear cleavage is widely observed[2-4].

Wang *et al*[13] considered BA as a kind of tumor associated with bronchioles, and bronchiole involvement can be found in almost all BAs. Upon careful observation, we also found the tumor to have expanded from bronchioles to the surrounding alveolar walls. Meanwhile we also observed the pulmonary lobular artery and bronchioles in these two cases as having local area; this formed a relatively robust basis for our diagnosis.

In typical morphologic cases, the double-layer structure is obvious, and ciliary cells and mucous cells are clearly recognizable on the lumen surface, eliminating the need for immunohistochemical examination. However, in our two cases, it was difficult to judge whether the basal cells were present, thus warranting immunohistochemical staining to visualize the tissue structure and cell type. In case 1, P40, P63, and CK5/6 staining were detected only in local area. Whereas in case 2, P63 and CK5/6 were expressed only in atypic cells, confounding our diagnosis. Many reports have indicated that the double-layer structure is essential in the diagnosis of BA; however, based on our understanding of the current cases and review of the related literature, we called these two lesions as monolayer BA lesions[14,15].

The presence of cellular atypia and the lack of the basal cell layer in monolayer BA lesions suggest their potential to transform into malignant tumors. These findings may reflect the continuous malignant transformation process of benign adenomas of the bronchial epithelium. Further large-scale studies of similar cases are required to learn whether monolayer BA lesions are accompanied by atypical bronchiolar epithelial hyperplasia, whether they are precancerous lesions and are similar to the atypical hyperplasia of the breast, and whether they will eventually become AIS or even invasive adenocarcinoma[15].

Although the distal type of bronchial adenoma typically has cilia and can be found to extend with the normal bronchioles, these characteristics are not easy to observe in intraoperatively frozen sections[6,16,17]. The differentiation of bronchial adenoma and

cancer requires immunohistochemistry-assisted diagnosis, which is not currently performed during the operation. Therefore, performing a differential diagnosis of bronchial adenoma and carcinoma using intraoperatively frozen sections during operation is difficult and challenging.

Although some studies have reported that ill-defined peripheral opacity and pseudocavities of a ground-glass lung nodule on CT differentiate BA from AIS or minimally invasive adenocarcinoma[18], these aspects are relative and not absolute. Thus, they provide some hints, but more comprehensive findings are required for differentiation of these lesions.

Kamata *et al*[19] identified cancer-driving gene mutations in CMPT, supporting the notion that these lesions are neoplastic rather than reactive or metaplastic. Unlike previous studies that primarily focused on EGFR and BRAF genes[5-9,20], we evaluated nine genes with susceptibility to cause BAs. Case 1 was negative for gene mutations in all genes. In case 2, HER2 gene mutation was found in the patient's DNA sample. Given the small number of samples in this case report, the significance of HER2 gene mutation needs to be further studied in a larger number of samples.

CONCLUSION

The results of our two cases are shown in Table 1. Although no meaningful conclusions could be drawn, these findings encourage further work in a larger sample size with control cases for better comparison. The current two cases were of monolayer BA lesions. Some unresolved questions remain to be answered to determine whether the lesion is a benign adenoma or part of the process of malignant transformation from benign adenoma of the bronchial epithelium. Furthermore, whether the lesions with atypical bilayer structures are similar to atypical hyperplastic lesions of the breast remains to be elucidated. In addition, whether these lesions can be called atypical BA and whether they are invasive precursor lesions need to be evaluated. Finally, future studies should examine whether HER2 gene mutation has diagnostic significance and value as a prognostic indicator in BA.

Regardless of whether the BA is benign or potentially malignant, simple surgical resection is the best choice for patient management. However, to determine BA, it is very important to use intraoperatively frozen sections. Performing a differential diagnosis of bronchial adenoma and carcinoma using intraoperatively frozen sections while the operation is underway is difficult and challenging. Hence, further study of this disease with a larger sample size and controls is required to draw meaningful conclusions.

71186_Auto_Edited-check.docx

ORIGINALITY REPORT

8%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	77 words — 2%
2	Ran Du, Feng Jiang, Zheng-Yan Wang, Yan-Qing Kang, Xiu-Yu Wang, Ying Du. "Pure large cell neuroendocrine carcinoma originating from the endometrium: A case report", World Journal of Clinical Cases, 2021 Crossref	44 words — 1%
3	www.wjgnet.com Internet	40 words — 1%
4	Qiang Zheng, Rongkui Luo, Yan Jin, Xuxia Shen, Ling Shan, Lei Shen, Yingyong Hou, Yuan Li. "So-called “non-classic” ciliated muconodular papillary tumors: a comprehensive comparison of the clinicopathological and molecular features with classic ciliated muconodular papillary tumors", Human Pathology, 2018 Crossref	17 words — < 1%
5	bmcresnotes.biomedcentral.com Internet	17 words — < 1%
6	Chunquan Liu, Huawei Li, Kaikai Xu, Shuai Song, Ying He, Xianqi Cai, Xiangyu Chu, Jiandong Yang, Yong Cui. "Multiple primary lung cancer versus intrapulmonary metastatic cancer: A case of multiple pulmonary nodules", Thoracic Cancer, 2018	14 words — < 1%

Crossref

7 L. Cao, Z. Wang, T. Gong, J. Wang, J. Liu, L. Jin, Q. Yuan. "Discriminating between bronchiolar adenoma, adenocarcinoma in situ and minimally invasive adenocarcinoma of the lung with CT", Diagnostic and Interventional Imaging, 2020 — 13 words — < 1%
Crossref

8 Jinchen Shao, Jiani C Yin, Hairong Bao, Ruiying Zhao, Yuchen Han, Lei Zhu, Xue Wu, Yang Shao, Jie Zhang. "Morphological, immunohistochemical, and genetic analyses of bronchiolar adenoma and its putative variants", The Journal of Pathology: Clinical Research, 2021 — 12 words — < 1%
Crossref

9 link.springer.com — 12 words — < 1%
Internet

10 www.mdpi.com — 12 words — < 1%
Internet

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY ON
EXCLUDE MATCHES < 12 WORDS