Genetic and immunohistochemical analyses of ciliated muconodular papillary tumors of the lung: A report of five cases

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Abstract
Ciliated muconodular papillary tumors are benign lesions located in the peripheral lung field. Recent studies revealed BRAF and epidermal growth factor receptor gene mutations and anaplastic lymphoma kinase gene rearrangement. Five ciliated muconodular papillary tumors were screened for the BRAF V600E and EGFR mutations via polymerase chain reaction. Immunohistochemical analysis was performed for the detection of the BRAF V600E and anaplastic lymphoma kinase proteins, as well as other markers including phosphorylated extracellular signal-regulated protein kinase. Three tumors (60%) harbored the BRAF V600E mutation. Immunohistochemical analysis confirmed this mutation in all of the tumor cell types. EGFR mutation and immunoactivity of the anaplastic lymphoma kinase protein were not detected. Phosphorylated extracellular signal-regulated protein kinase was negative both in the cytoplasm and nucleus of the BRAF V600E–positive tumors. Mucin 1, mucin 4, thyroid transcription factor 1, and cytokeratin 7 were positive, and mucin SAC was partially positive, whereas napsin A and cytokeratin 20 were negative. Ciliated muconodular papillary tumor may originate from the terminal bronchioles, and the status of ERK activation reflects its benign behavior.

Keywords
BRAF V600E, ciliated muconodular papillary tumor, immunohistochemical analysis, lung, phosphorylated extracellular signal-regulated protein kinase

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Introduction
A ciliated muconodular papillary tumor (CMPT) is characterized by papillary proliferation of ciliated columnar cells, goblet cells, and basal cells. Of the approximate 30 cases of CMPTs reported in the English literature,1–9 no recurrence or metastasis has been reported. In a study of 10 CMPTs, Kamata et al.3 revealed that 50% harbored a BRAF mutation, and 30% had an epidermal growth factor receptor (EGFR) mutation. Other studies reported CMPTs with AKT mutations, anaplastic lymphoma kinase (ALK) rearrangements, and KRAS mutations.2,5,6,9 These results indicate that a CMPT is a neoplastic lesion.

Extracellular signal-regulated kinase (ERK) is a component of the mitogen-activated protein kinase (MAPK) pathway and activated by phosphorylation and nuclear translocation. ERK activation has been suggested to play a role in the pathogenesis and progression of various cancers. The BRAF V600E mutation is reported to activate the MAPK pathway and promote cell proliferation. A previous study reported a poorer prognosis of ERK-activated colon cancer than of colon cancer without ERK activation.10 However, to the best of our knowledge, no report has yet addressed the status of ERK activation in CMPT.

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In this study, \textit{BRAF} V600E and \textit{EGFR} mutations were screened in five CMPTs resected at our hospital. Immunohistochemical (IHC) analysis of the \textit{BRAF} V600E mutation and \textit{ALK} was also performed. Moreover, immunostaining of phosphorylated extracellular signal-regulated kinase (p-ERK) was performed to reveal the role of the MAPK pathway in the pathogenesis of CMPT. Tumor origin was also estimated by IHC staining of mucin core proteins and diagnostic marker proteins of lung cancer. This study is conducted independently and does not constitute any other larger studies.

**Case section**

**Patient characteristics**

The characteristics of five patients (2 male, 3 females) are shown in Table 1. All tumors were single and less than 18 mm in diameter. No recurrence or metastasis was observed during follow-up examinations conducted from 0.5 to 6 years. Three patients had a history of malignancy.

**Histological analysis of CMPT**

All tumors consisted of ciliated columnar cells, mucinous cells, and basal cells arranged in papillary and glandular structures (Figure 1(a) and (b)), consistent with the features of CMPTs noted in previous reports. A transitional zone from normal bronchiolos with 0.2 mm diameters to CMPT was observed in patient 3 (Figure 1(e)).

**IHC analysis of \textit{BRAF} V600E, ALK, and p-ERK**

Immunostaining for \textit{BRAF} V600E was positive in tumors from patients 3, 4, and 5 (Figure 1(c) and (d) and Table 2). All three types of tumor cells were stained. The cilia of adjacent bronchiolos were also stained. In the transitional zone from normal bronchiolos to CMPT, cytoplasmic staining of CMPT contrasted with that of the bronchiolar epithelium (Figure 1(f) and (g)).

IHC staining of all five tumors were negative for the ALK protein.

Staining for p-ERK was negative in both the cytoplasm and nucleus of the \textit{BRAF} V600E–positive tumor cells (Figure 2(a) and Table 2). However, in \textit{BRAF} V600E–negative tumors, some nuclei of the mucinous cells were positive for p-ERK (Figure 2(b) and Table 2).

**IHC analysis of mucin core proteins and lung cancer markers**

The results of IHC analysis for mucin core proteins and lung cancer–related markers are shown in Table 2. All tumors were positive for MUC1 and MUC4, whereas some columnar and mucinous cells were positive for MUC5AC. The tumors were also positive for thyroid transcription factor 1 (TTF-1) and cytokeratin 7 (CK7) but negative for napsin A and cytokeratin 20 (CK20).

**Gene mutation analysis by polymerase chain reaction**

The DNA extracted from dissected tumors was screened for the \textit{BRAF} V600E mutation. Three tumors that were positive for the \textit{BRAF} V600E mutation by IHC analysis harbored the \textit{BRAF} V600E mutation (patients 3, 4, and 5; Figure 3). Isolated bronchiolos of patient 5 were also examined by laser capture microdissection, which showed that all were negative for the \textit{BRAF} V600E mutation (data not shown). \textit{EGFR} mutations were also screened using extracted DNA from formalin-fixed, paraffin-embedded tissues according to the polymerase chain reaction-based method described previously. All tumors were negative for \textit{EGFR} mutations.

**Discussion**

In this study, three of five CMPTs harbored the \textit{BRAF} V600E mutation, whereas the others were negative for the \textit{BRAF} V600E mutation, \textit{EGFR} mutations, and \textit{ALK} gene rearrangements. A past study reported that 50% of CMPTs harbored a \textit{BRAF} mutation, but no mutations of 50 screened genes were correlated to cell proliferation. In this study, screening was limited to the \textit{BRAF} V600E mutation. \textit{EGFR} mutations, including one detected in a past study, were also screened. Other than ALK expression by IHC analysis, no other genetic analysis was performed. Therefore, tumors with no detected mutations in this study may have mutations of \textit{BRAF} and/or other genes, such as \textit{AKT} and \textit{KRAS}, as previously reported.

Other gene mutations may coexist even in tumors positive for the \textit{BRAF} V600E mutation.

IHC analysis with the VE1 monoclonal antibody revealed that the cytoplasm and cilia of CMPTs and the normal bronchiolar epithelium were positive for the \textit{BRAF} V600E mutation.

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**Table 1. Patient characteristics.**

| No. | Age | Sex | Tumor location | Tumor diameter (mm) | Follow-up duration (years) | Other tumors |
|-----|-----|-----|----------------|--------------------|---------------------------|--------------|
| 1   | 71  | M   | RLL            | 9                  | 0.5                       | None         |
| 2   | 72  | F   | UUL            | 9                  | 1                         | Thyroid papillary carcinoma |
| 3   | 73  | F   | LLL            | 12                 | 6                         | None         |
| 4   | 64  | M   | LLL            | 8                  | 5                         | Laryngeal carcinoma   |
| 5   | 56  | F   | RLL            | 18                 | 2                         | Glioblastoma, uterine tumor |

LUL: left upper lobe; LLL: left lower lobe; RLL: right lower lobe.
However, genetic analysis to identify other mutations of the bronchiole was not performed. A previous report indicated that the VE1 antibody cross-reacted with the dynein protein in cilia, including that in the bronchial epithelium. Therefore, the positive staining of cilia was considered to be a false positive. Meanwhile, cytoplasmic staining was clearly stronger for the CMPTs than for the bronchial epithelium at the transitional zone, suggesting that the \( \text{BRAF} \) \(^{V600E} \) mutation can be identified in CMPTs by cytoplasmic staining.

Histological analysis of CMPTs revealed a papillary structure originating from the central airway. A transitional zone of normal bronchioles (0.2 mm in diameter) to CMPT was also observed in patient 2. Approximately, all cells were positive for MUC1 and MUC4, and partially positive for MUC5AC. The former two proteins are reportedly expressed in the epithelium of the central and peripheral airways, whereas the expression of the latter protein is decreased in peripheral bronchioles less than 1 mm in diameter. All CMPTs were negative for napsin A, which is known to be expressed in type II alveolar epithelium. \( \text{BRAF} \) mutations are reported to exist in less than 3% of lung adenocarcinomas. The prevalence of \( \text{BRAF} \) mutations of

**Figure 1.** Histological analysis of CMPT and IHC analysis of the \( \text{BRAF} \) \(^{V600E} \) mutation. Ciliated columnar cells, mucinous cells, and basal cells formed papillary and glandular structures (a, b). IHC analysis of \( \text{BRAF} \) \(^{V600E} \) with VE1 antibody in patients 4 and 1 (c, d). (b) A transitional zone between the normal bronchioles and tumor was observed in patient 3 (e). Cytoplasmic staining was stronger for CMPT than for the normal bronchioles in the transitional zone of patient 3 (f) and (g): high magnification.

**Table 2.** Immunohistochemical analysis.

| No. | MUC1 | MUC2 | MUC4 | MUC5AC | MUC6 | TTF-1 | Napsin A | p40 | CK7 | CK20 | \( \text{BRAF} \) \(^{V600E} \) | p-ERK |
|-----|------|------|------|--------|------|-------|----------|-----|-----|------|-----------------|------|
| 1   | ++   | –    | + (c,m) | + (c) | –    | + +   | –        | + (b) | + + | –    | –               | + (m) |
| 2   | +    | –    | + (c,m) | + (m) | –    | + +   | –        | + (b) | + + | –    | –               | + (m) |
| 3   | +    | –    | + (c,m) | + (m) | –    | + +   | –        | + (b) | + + | –    | +               | –    |
| 4   | ++   | –    | + (c,m) | + (c) | –    | + +   | –        | + (b) | + + | –    | +               | –    |
| 5   | ++   | –    | + (c,m) | + (c,m) | –    | + +   | –        | + (b) | + + | –    | +               | –    |

TTF-1: thyroid transcription factor 1; MUC: mucin; CK: cytokeratin; p-ERK: phosphorylated extracellular signal-regulated kinase; b: basal cells; c: ciliated columnar cells; m: mucinous cells; –: negative; +: mild; ++: moderate; +++: diffuse.
adenocarcinoma is extremely low compared with that of CMPT in the present and a previous study. According to the results of IHC and gene mutation analyses, a CMPT may originate from peripheral bronchioles, which is different from lung adenocarcinoma.

The status of ERK activation in other cancers reportedly varies. In contrast, a previous study reported that colon cancer cells with a BRAF mutation had a lower level of nuclear positivity and higher level of cytoplasmic positivity for p-ERK compared with those of colon cancer cells with wild-type BRAF. Dual-specificity phosphatases participate in a negative feedback mechanism responsible for the dephosphorylation of nuclear p-ERK. This system may explain the lack of nuclear staining of p-ERK in colon cancer cells. In this study, p-ERK was negative in the cases in which BRAF V600E was detected suggesting the involvement of other mechanisms in the de-activation of the MAPK cascade in CMPTs. In any case, the status of p-ERK activation may explain the benign nature of a CMPT.

### Conclusion

We presented five CMPTs and three of them harbored the BRAF V600E mutation. Histological and IHC analyses indicated a bronchiolar origin of CMPTs. Negative staining of nuclear p-ERK may reflect the restricted ability of proliferation signaling pathways.

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### References

1. Ishikawa M, Sumitomo S, Imamura N, et al. Ciliated mucinous papillary tumor of the lung: report of five cases. J Surg Case Rep 2016; 2016(8): rjw144.
2. Jin Y, Shen X, Shen L, et al. Ciliated mucinous papillary tumor of the lung harboring ALK gene rearrangement: case report and review of the literature. Pathol Int 2017; 67(3): 171–175.
3. Kamata T, Sunami K, Yoshida A, et al. Frequent BRAF or EGFR mutations in ciliated mucinous papillary tumors of the lung. J Thorac Oncol 2016; 11(2): 261–265.
4. Kon T, Baba Y, Fukai I, et al. Ciliated mucinous papillary tumor of the lung: a report of five cases. Pathol Int 2016; 66(11): 633–639.
5. Liu L, Aesif SW, Kipp BR, et al. Ciliated mucinous papillary tumors of the lung can occur in Western patients and show mutations in BRAF and AKT1. Am J Surg Pathol 2016; 40(12): 1631–1636.
6. Taguchi R, Higuchi K, Sudo M, et al. A case of anaplastic lymphoma kinase (ALK)-positive ciliated mucinous papillary tumor (CMPT) of the lung. Pathol Int 2017; 67(2): 99–104.
7. Chuang HW, Liao JB, Chang HC, et al. Ciliated mucinous papillary tumor of the lung: a newly defined peripheral...
pulmonary tumor with conspicuous mucin pool mimicking colloid adenocarcinoma: a case report and review of literature. *Pathol Int* 2014; 64(7): 352–357.
8. Sato S, Koike T, Homma K, et al. Ciliated muconodular papillary tumour of the lung: a newly defined low-grade malignant tumour. *Interact Cardiovasc Thorac Surg* 2010; 11(5): 685–687.
9. Udo E, Furusato B, Sakai K, et al. Ciliated muconodular papillary tumors of the lung with KRAS/BRAF/AKT1 mutation. *Diagn Pathol* 2017; 12(1): 62.
10. Yeh JJ, Routh ED, Rubinas T, et al. KRAS/BRAF mutation status and ERK1/2 activation as biomarkers for MEK1/2 inhibitor therapy in colorectal cancer. *Mol Cancer Ther* 2009; 8(4): 834–843.
11. Benlloch S, Botero ML, Beltran-Alamillo J, et al. Clinical validation of a PCR assay for the detection of EGFR mutations in non–small-cell lung cancer: retrospective testing of specimens from the EURTAC trial. *PLoS ONE* 2014; 9(2): e89518.
12. Jones RT, Abedalthagafi MS, Brahmandam M, et al. Cross-reactivity of the BRAF VE1 antibody with epitopes in axonemal dyneins leads to staining of cilia. *Mod Pathol* 2015; 28(4): 596–606.
13. Copin MC, Devisse L, Buisine MP, et al. From normal respiratory mucosa to epidermoid carcinoma: expression of human mucin genes. *Int J Cancer* 2000; 86(2): 162–168.
14. Paik PK, Arcila ME, Fara M, et al. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J Clin Oncol* 2011; 29(15): 2046–2051.
15. Schmitz KJ, Wohlschlaeger J, Alakus H, et al. Activation of extracellular regulated kinases (ERK1/2) but not AKT predicts poor prognosis in colorectal carcinoma and is associated with k-ras mutations. *Virchows Arch* 2007; 450: 151–159.