BRIEF REPORT

Frequent *BRAF* or *EGFR* Mutations in Ciliated Muconodular Papillary Tumors of the Lung

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ABSTRACT

Introduction: Ciliated muconodular papillary tumors (CMPTs) are recently characterized, rare peripheral nodules of the lung. These small tumors are histologically comprised of a vaguely organized mixture of nonatypical ciliated columnar cells, mucous cells, and basal cells, and consistently follow a benign clinical course. However, the histogenesis of CMPTs remains uncertain.

Methods: We performed detailed genomic analyses of 10 archived CMPT cases, using next-generation sequencing and high-resolution melting analysis.

Results: Mutations were identified in eight of the 10 cases (80%); four cases harbored the *BRAF*-V600E mutation, one case harbored the *BRAF*-G606R mutation, and three cases harbored deletions in exon 19 of *EGFR*. All of the deletions in *EGFR* were of the E746-T751/S752V subtype.

Conclusions: The high prevalence of driver gene mutations in CMPTs supports the notion that these lesions are neoplastic rather than reactive or metaplastic.

Keywords: *BRAF*; Ciliated muconodular papillary tumors; *EGFR*; Histogenesis; Next-generation sequencing

Introduction

Ciliated muconodular papillary tumors (CMPTs) are rare peripheral nonendobronchial lung nodules that have recently been characterized. These small nodules are typically located in the peribronchiolar region, and exhibit an overall papillary or glandular architecture. CMPTs consist of a vaguely organized mixture of nonatypical ciliated columnar cells, mucous cells, and basal cells, and are typically enveloped with abundant mucus. Because of the focal stromal destruction and complex architecture—which includes papillary, micropapillary, and focally discontinuous lepidic growths—these lesions may be confused with adenocarcinomas. However, CMPTs consistently follow a benign clinical course, even after limited surgery, and an appropriate differential diagnosis is critical for appropriate patient management.[1] For example, cilia are not usually observed in pulmonary adenocarcinoma, and the presence of cilia and/or terminal plates is one of the most reliable findings to support a benign diagnosis.[2,3] However, exceptional cases of ciliated adenocarcinoma in the lung have been reported.[4]

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The histogenesis of CMPT remains poorly understood. For example, the organized growth pattern and consistent peribronchiolar localization may indicate a metaplastic phenomenon. However, the complex architecture, focal stromal destruction, and autonomous enlargement that have been observed in a few cases indicate a neoplastic quality. Therefore, we undertook next-generation sequencing and high-resolution melting analyses (HRMA) of 10 well-annotated cases of CMPTs to clarify their histogenesis.

Methods

Patients and Clinicopathological Analysis

This study was approved by the institutional review board of the National Cancer Center Hospital (Tokyo, Japan). A total of 10 CMPT cases were identified in our institution between 2006 and 2014. Their clinical and pathological records were retrospectively reviewed, and we performed histological analyses of the resected specimens.

DNA Preparation

We analyzed 10 tumor specimens, including 4 fresh frozen and 6 formalin-fixed, paraffin-embedded (FFPE) tissue samples. Genomic DNA was extracted from the fresh frozen tissues using the QIAamp DNA mini Kit (QIAGEN, Hilden, Germany), and from the FFPE tissues using the QIAamp DNA FFPE Tissue Kit (QIAGEN). DNA was quantified using the Qubit Fluorometer (Life Technologies, Carlsbad, CA).

Target Deep Sequencing of Mutation Hotspots in 50 Cancer-Related Genes

Ten nanograms of DNA were subjected to library preparation for the Ion Ampliseq Cancer Hotspot Panel v2 (Life Technologies), which can detect mutations at 2790 hot spots in 50 cancer-related genes (Table 1). The library DNAs were prepared by amplifying the targeted regions using multiplex polymerase chain reaction (PCR), which was followed by adopter DNA ligation. Concentrations of the library DNA were evaluated via quantitative real-time polymerase chain reaction studies using the Ion Library Quantitation Kit (Life Technologies). Sequencing was performed using the Ion Proton platform (Life Technologies), and mutations were identified using Torrent Suite software (Life Technologies).

Table 1. Cancer Hotspot Panel of 50 Cancer-Related Genes

ABL1	*EGFR*	*GNA11*	*KRAS*	*PTPN11*
AKT	*ERBB2*	*GNAQ*	*MET*	*RB1*
ALK	*ERBB4*	*HNF1A*	*MLH1*	*RET*
APC	*EZH2*	*HRAS*	*MPL*	*SMAD4*
ATM	*FBXW7*	*IDH1*	*NOTCH1*	*SMARCB1*
BRAF	*FGFR1*	*IDH2*	*NPM1*	*SMO*
CDH1	*FGFR2*	*JAK2*	*NRAS*	*SRC*
CDKN2A	*FGFR3*	*JAK3*	*PDGFRA*	*STK11*
CSF1R	*FLT3*	*KDR*	*PIK3CA*	*TP53*
CTNNB1	*GNAS*	*KIT*	*PTEN*	*VHL*

Confirmatory Analyses

To confirm the *BRAF* and *EGFR* mutations, we performed HRMA (Idaho Technology, Salt Lake City, UT), based on a previously reported method.[5] We also performed immunohistochemistry using a monoclonal antibody to BRAF V600E (1:200 dilution; clone VE1; Spring Bioscience, Pleasanton, CA), and the results were estimated separately for each of the three epithelial components (i.e., ciliated columnar cells, mucous cells, and basal cells). Cytoplasmic staining was considered a positive result.

Results

Clinical Findings

Detailed clinicopathological data for the identical 10 cases have been published separately.[1] All tumors showed semiorganized peribronchiolar growth that consisted of tripartite cellular elements, including ciliated columnar cells, mucous cells, and basal cells (Fig. 1). None of the patients experienced local recurrence or distant metastasis during a mean follow-up of 43 months (range, 2–88 months).

Target Deep Sequencing of Mutation Hotspots

We conducted next-generation sequencing at a mean depth of ×2237 (range, ×138–12,650) to search for mutations at hotspots in 50 cancer-related genes from

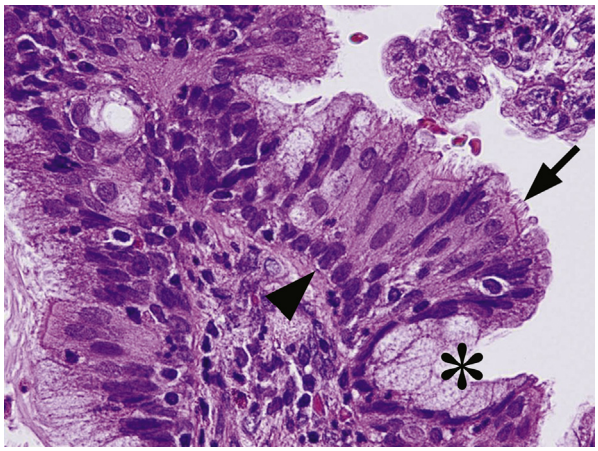

Figure 1. A representative image of ciliated muconodular papillary tumors with hematoxylin-eosin staining. The epithelial components of the lesions consisted of a mixture of ciliated columnar cells (arrow), mucous cells (asterisk), and basal cells (arrowhead).

the 10 CMPT tissue specimens. The average total number of raw reads per sample was 1,022,275 (range, 447,913–1,910,793), and the mean rate of uniquely mapped reads was 92.2% (range, 79.0%–95.9%). One or more mutations were detected in 8 of the 10 cases (80%; Table 2). The mutated genes consisted of *BRAF* (50%), *EGFR* (30%), *PTPN11* (20%), *CTNNB1* (10%), *IDH1* (10%), and *TP53* (10%). The mutations in *BRAF* were two types of missense mutations (V600E in four cases and G606R in one case), and all three *EGFR* mutations were the same in frame deletion (delE746-T751/S752V). All *BRAF* and *EGFR* mutations were validated using HRMA. The *BRAF* and *EGFR* mutations were mutually exclusive among the specimens (Fig. 2).

Immunohistochemistry

In the four cases with the *BRAF* V600E mutation, all three epithelial components (i.e., ciliated columnar cells, mucous cells, and basal cells) showed similar cytoplasmic staining for BRAF V600E. This finding suggests that all 3 elements were neoplastic components (Fig. 3). As expected, the BRAF V600E–specific antibody reacted strongly with the cilia of the columnar cells (the internal positive control).[6]

Discussion

In this study, we identified driver gene mutations in most CMPTs, and provided evidence that these enigmatic lesions are indeed neoplastic processes.

Five of the 10 CMPTs (50%) harbored *BRAF* mutations, including four V600E mutations and one G606R mutation. Mutations in *BRAF* are a common driver in many different types of benign and malignant tumors in humans[7,8]; the most common type is the V600E substitution. *BRAF* is rarely (3%) mutated in lung adenocarcinomas, and this mutation is even rarer (1.3%) among Asian patients.[9] Therefore, the high incidence of *BRAF* mutations in CMPTs presents a contrast with the incidence in lung adenocarcinomas. In addition, our histological findings exclude a clear relationship between CMPTs and adenocarcinomas.[1] In addition, *BRAF*-mutated lung adenocarcinomas typically exhibit aggressive features, such as micropapillary, acinar, and solid growth patterns, and CMPT-like morphology has not been documented in our cohort or in a previously reported cohort.[9] Moreover, none of our *BRAF* mutant CMPTs exhibited worrisome histological findings.

Similarly, the *EGFR* mutations in three of our CMPTs do not necessarily link these lesions to adenocarcinomas. Although exon 19 deletions (Del-19s) are one of the most common alterations in lung adenocarcinomas, the specific Del-19 that we found in our CMPTs is extremely rare in lung adenocarcinomas. According to the Somatic Mutations in EGFR Database, the most frequent Del-19 in lung adenocarcinomas is delE746-A750 (28.89%), followed by delL747-P753insS (2.49%) and delL747-A750insP (1.73%).[10] However, the *EGFR* deletion in three of our CMPTs was delE746-T751/S752V, and this deletion is extremely rare in lung adenocarcinomas (only one reported case).[10] In addition, that particular case likely did not involve a CMPT, because cilia were not documented. Therefore, CMPT-like morphology has never been documented in *EGFR*-mutated cancers, and the presence of mucous cells is rare in *EGFR*-mutated adenocarcinomas. Moreover, none of the *EGFR* mutant CMPTs in our cohort exhibited malignant histological findings.

Table 2. Gene Mutations Detected in 10 CMPTs

Case No.	Age (y)	Sex	Smoking	Size (cm)	Tissue Type	Driver Mutation (Cosmic ID)	Other Mutations	Mutation Allele Frequency
1	61	F	N	0.6	Frozen	*BRAF*-V600E (COSM476)	-	-
2	56	M	Y	1.1	FFPE	*BRAF*-V600E (COSM476)	-	-
3	61	M	N	1.0	FFPE	*BRAF*-G606R (COSM27640)	*IDH1*-G123R (COSM96922), *CTNNB1*-D32N (COSM5672), and *PTPN11*-E76K (COSM13000)	6 9 8
4	60	F	N	1.5	Frozen	*EGFR*-ex19del E746-T751/S752V (COSM12384)	-	-
5	62	F	N	1.3	Frozen	*EGFR*-ex19del E746-T751/S752V (COSM12384)	-	-
6	66	M	N	0.7	FFPE	*EGFR*-ex19del E746-T751/S752V (COSM12384)	*PTPN11*-P491L (COSM13034) and *TP53*-L289F(COSM45446)	16 7
7	75	M	Y	0.6	FFPE	Not detected	-	-
8	57	M	Y	1.2	Frozen	Not detected	-	-
9	78	M	Y	0.9	FFPE	*BRAF*-V600E (COSM476)	Not tested	-
10	63	M	Y	1.1	FFPE	*BRAF*-V600E (COSM476)	Not tested	-

F, female; FFPE, formalin-fixed paraffin-embedded specimens; M, male; N, no; Y, yes.

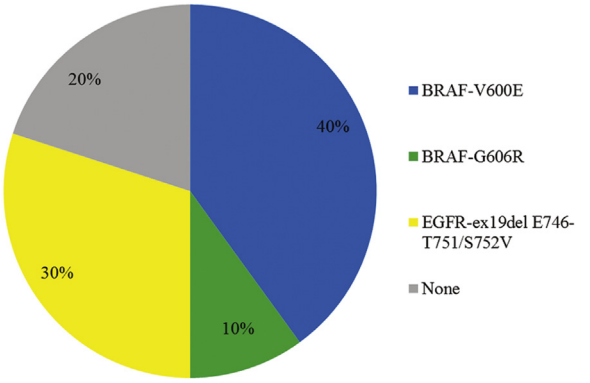

Figure 2. The results of the mutation analyses for 10 ciliated muconodular papillary tumors.

It is notable that the presence of driver gene alterations in CMPTs does not dictate their clinical behavior, because gene mutations can be present both in benign and malignant lesions. For example, *BRAF* mutations have been found in benign lesions, such as melanocytic nevi, Langerhans cell histiocytosis, biliary adenomas, and pilocytic astrocytomas.[11,12] In addition, the *EGFR* mutation has been detected in atypical adenomatous hyperplasia.[13] However, none of the reported CMPT cases, including our present series, have metastasized to the local lymph nodes or distant sites, and none recurred after surgery. The benign nature of CMPTs is also implied by the bland cytology, the lack of mitotic figures, and the invariable presence of ciliated and basal cell populations within the tumor. Nevertheless, larger studies with longer follow-ups are necessary to accurately determine the course of CMPTs.

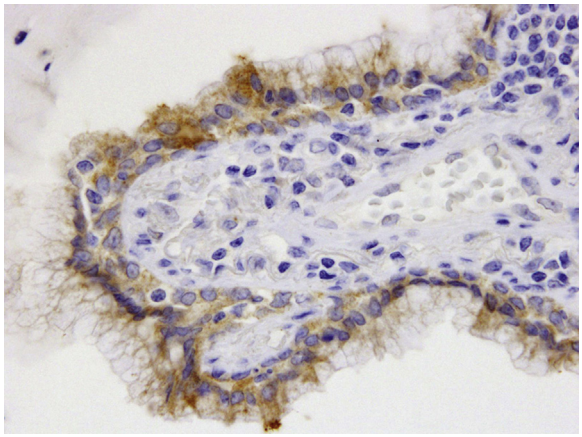

Figure 3. Immunohistochemical analyses of the *BRAF*-V600E ciliated muconodular papillary tumors. Each of the three epithelial components (i.e., ciliated columnar cells, mucous cells, and basal cells) exhibited cytoplasmic staining for *BRAF*-V600E. The monoclonal antibody also strongly cross-reacted with the cilia of the columnar cells (the internal positive control).

Immunohistochemical analysis with the BRAF V600E–specific antibody revealed that all three epithelial components (i.e., ciliated cells, mucous cells, and basal cells) were equally positive for this antibody. This finding supports the theory that each of these tripartite elements constitutes a neoplastic component, at least in these four CMPTs. This staining also eliminates the remote possibility that florid metaplastic changes obscured the presence of a minute *BRAF*-mutant adenocarcinoma.

Our discovery of *BRAF* and *EGFR* mutations in most of our CMPT cases may also help explain the relationship between rare benign lesions in the lung. For example, several groups of researchers have reported unusual peripheral lung lesions that are histologically similar (if not identical) to CMPTs, using names such as "solitary peripheral ciliated glandular papillomas," "mucinous adenomatous hyperplasia," and "peripheral pulmonary papillary/glandular neoplasms with ciliated cells."[14,15] Similarly, the histologic features of mixed papillomas resemble those of CMPTs,[2] although these two tumors can be differentiated by at least their locations (central versus peripheral) and their associations with the bronchial lumens (endobronchial versus nonendobronchial). Therefore, *BRAF* and *EGFR* mutational assays in these benign tumors may clarify their nosological relationship with CMPTs.

In conclusion, we have identified frequent driver mutations of *BRAF* or *EGFR* in most of our 10 CMPT cases. These data support the notion that these lesions are neoplastic rather than reactive or metaplastic.

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