Case Report

Ciliated muconodular papillary tumor of the lung harboring *BRAF* V600E mutation and p16^{INK4a} overexpression without proliferative activity may represent an example of oncogene-induced senescence

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Abstract: Ciliated muconodular papillary tumor (CMPT) is a rare peripheral lung tumor that shows puzzling histologic features encompassing metaplastic and neoplastic nature. This type of tumor is occasionally misdiagnosed as lung adenocarcinoma clinically and pathologically, and its pathogenic mechanism has not been well characterized. We experienced a case of CMPT in a 73-year-old male and performed targeted deep sequencing to characterize its molecular features. The tumor was an ill-defined, subpleural, and non-endobronchial nodule showing glandular and papillary proliferation of mucous cells, ciliated columnar cells, and basal cells without any cytologic atypia. Abundant intra-alveolar mucin surrounded the main lesion. The patient was well without recurrence throughout 36 months of follow-up. Our case harbored *BRAF* V600E mutation and strongly expressed p16^{INK4a} without proliferative activity, representing senescence and indolent biologic behavior. Overall, the results of this study indicate that *BRAF* V600E mutation might be the driver for tumorigenesis of CMPT and eventually leads to oncogene-induced senescence of this tumor.

Keywords: Ciliated muconodular papillary tumor (CMPT); *BRAF*; p16^{INK4a}; senescence; deep sequencing

Submitted Apr 19, 2017. Accepted for publication Nov 13, 2017.
doi: 10.21037/jtd.2017.11.120
View this article at: http://dx.doi.org/10.21037/jtd.2017.11.120

Introduction

Ciliated muconodular papillary tumor (CMPT) is a recently defined lung tumor that shows a papillary or glandular proliferation of ciliated columnar cells, mucous cells, and basal cells surrounded by intra-alveolar mucin pools in the peripheral lung (1-6). This type of tumor has bland cytologic features and all cases reported to date have consistently shown an indolent clinical course. Recently, CMPTs have been encountered more frequently as the use of the low-dose computed tomography (CT) for screening of lung cancer has increased. Because CMPT is usually seen in the subpleural lung parenchyma as a solitary pulmonary nodule, it might be misdiagnosed as lung cancer clinically and radiologically (2).

Although the organized growth pattern with various cellular components including ciliated cells and basal cells, ill-defined nodular lesion, and common occurrence in the peribronchiolar area favor a metaplastic nature, solitary presentation, characteristic histological features and adenomatous proliferation of mucous cells suggest that this tumor is a neoplasm. Recently, the presence of driver mutations in *BRAF*, *EGFR* or *KRAS* in a subset of CMPT was reported (3-5) and this finding strongly suggests that CMPT is a neoplasm rather than a metaplastic process.

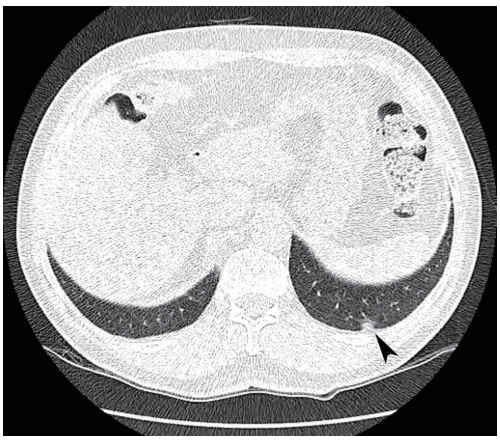

Figure 1 Radiologic finding of ciliated muconodular papillary tumor (CMPT). Low-dose chest computed tomography (CT) reveals a 9-mm-sized, solitary, irregular ground glass nodule (arrowhead) just beneath the pleura in posterior basal segment of left lower lobe.

However, the role of oncogenic mutations in the pathogenic mechanism of CMPT has not been evaluated yet.

Here, we report a case of CMPT harboring *BRAF* V600E mutation as well as $p16^{INK4a}$ overexpression and no proliferative activity and propose that oncogene-induced senescence (OIS) might be the pathogenic mechanism of CMPT.

Case presentation

A 72-year-old man visited our hospital due to an incidentally identified lung mass. The patient is a former smoker with a 30-pack-year smoking history, but had quit 4 years ago. He does not have any medical problems such as diabetes mellitus, hypertension or pulmonary tuberculosis. Low-dose chest CT was performed for routine health examination and a pleural based, 9-mm ground glass nodule was detected in the posterior basal segment of the left lower lobe (*Figure 1*). On follow-up CT performed after 6 months, the size of the nodule had not increased. However, it showed mildly increased ^{18}F-fluorodeoxyglucose (FDG) activity on positron emission tomography (PET)-CT. Clinically lung cancer was suspected and video-assisted thoracoscopic wedge resection was performed for pathological diagnosis.

Grossly, an ill-defined small mucoid nodule was seen in the subpleural area without pleural retraction. On low-power examination, the tumor was composed of nodular mucous cell proliferation surrounded by an intra-alveolar mucin pool, mimicking mucinous adenocarcinoma (*Figure 2A*). The tumor showed adenomatous proliferation and focal broad papillary fronds of bland-looking epithelial cells (*Figure 2B*). Mucous cell was the predominant cell type in adenomatous proliferation. The luminal surface of papillary fronds and some glands were composed of organized growth of three cellular components including mucous cells, ciliated columnar cells and underlying basal cells (*Figure 2C*). Ciliated cells between mucous cells were pseudostratified and protruding into the space. The epithelial cells did not show any nuclear atypia, mitotic figure, or necrosis. Immunohistochemically, all types of epithelial cells were positive for cytokeratin 7. P63 immunostaining highlighted continuous lining of basal cells beneath the columnar cells. A subset of ciliated columnar cells and basal cells was positive for TTF-1. Ki-67-positive epithelial cells were not seen, suggesting no proliferative activity of the tumor. The tumor cells were strongly positive for $p16^{INK4a}$ (*Figure 2D*), whereas few of them showed weak positivity for p53 immunostaining. Targeted deep sequencing with the Ion Ampliseq^{TM} Comprehensive Cancer Panel (Life Technologies, Carlsbad, CA, USA) demonstrated *BRAF* V600E point mutation (mutant allele frequency: 13.0%) (*Figure 3*), which was confirmed with a PNAClamp^{TM} *BRAF* mutation kit (Panagene, Daejeon, South Korea). High risk-human papillomavirus (HPV) was not detected by real time PCR assay (HPV PANA RealTyper^{TM}, Panagene). Post-surgical complication was not reported, and the patient has been doing well for 36 months of follow-up.

Discussion

In 2002, Ishikawa first proposed the new entity of CMPT in the peripheral lung showing a papillary proliferation of ciliated columnar cells, mucous cells and basal cells surrounded by a mucin pool filling the adjacent alveoli (1). A recent collective review by Kamata *et al.* encompassing ten cases of CMPT delineated this tumor as a distinct entity with specific clinicopathologic features and following an indolent clinical course (6). Recently, researchers demonstrated the oncogenic driver mutations in a considerable proportion of CMPT, suggesting the neoplastic nature of this entity (3-5). Kamata *et al.* reported five cases of CMPT with *BRAF* mutations; V600E in four and G606R in one (3). Liu *et al.* and Udo *et al.* also demonstrated *BRAF* V600E (4,5). Other mutations, such as *EGFR* (delE746_T751/S752V), *KRAS* (G12D), *PTPN11*

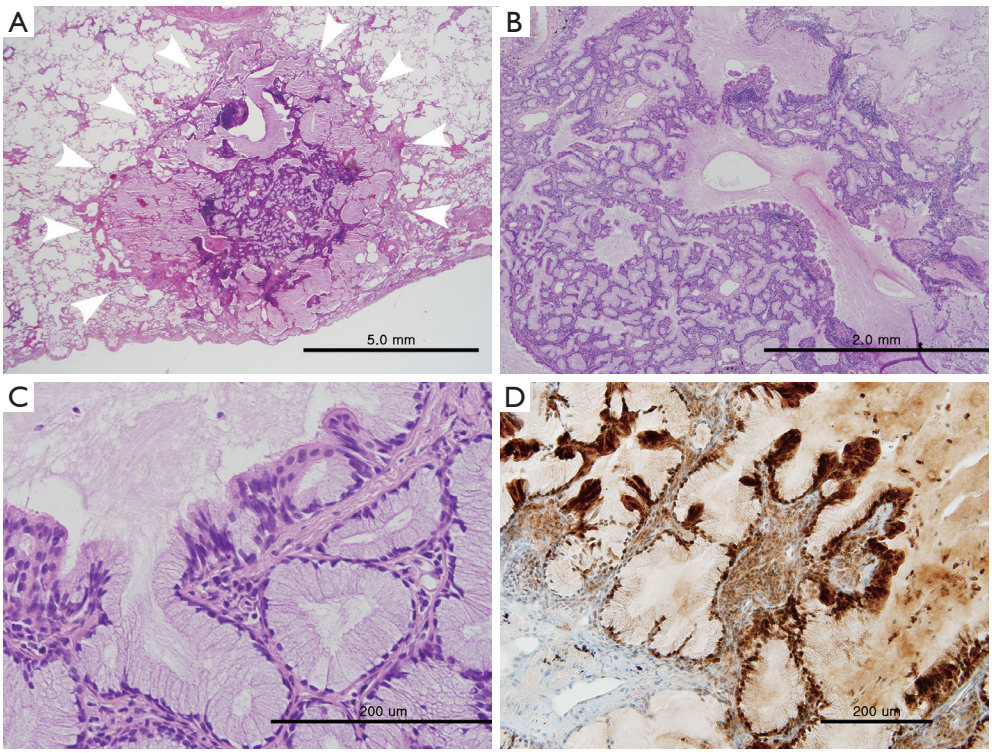

Figure 2 Histological and immunohistochemical features of CMPT. (A) An ill-defined nodular proliferation of glandular cells surrounded by irregularly distended air space filled with mucin is seen in the subpleural area (arrowhead) (hematoxylin and eosin stain); (B) the tumor is predominantly composed of bland-looking mucous cells forming glandular and multifocal vague papillary structure (hematoxylin and eosin stain); (C) the papillary structure shows organized growth of three cellular components including mucous cells, ciliated columnar cells and underlying basal cells (Hematoxylin and eosin stain); (D) all three types of tumor cells were strongly positive for $p16^{INK4a}$ (immunohistochemical staining).

(P491L), *CTNNB1* (D32N), *IDH1* (G123R), *AKT1* (E17K), and *TP53* (L289F) have also been detected (3-5). However, the role of oncogenic driver mutations on the pathogenesis of CMPT has not been discussed yet. Here we report a case of CMPT showing characteristic histological findings and harboring distinct molecular features with *BRAF* V600E mutation, $p16^{INK4a}$ overexpression, and no proliferative activity.

OIS is a robust and sustained cell cycle arrest induced by oncogenic mutation. The driver oncogenes which are commonly associated with OIS are *BRAF*, *HRAS* and *KRAS* (7). The $p16^{INK4a}$ is a tumor suppressor inducing cell cycle arrest, and has protective role against *BRAF* V600E-driven proliferation. It is upregulated in senescent cells (7) and one of the markers of OIS (8), although factors other than $p16^{INK4a}$ also contribute to the senescence caused by *BRAF* V600E (7).

BRAF is a well-known driver oncogene that is mutated in many types of malignant tumors such as malignant melanoma, thyroid papillary carcinoma and colonic adenocarcinoma, and V600E point mutation is the most common type. Interestingly, *BRAF* V600E mutation is frequently found in various types of benign tumors. For example, most acquired melanocytic nevi and a subset of colonic serrated polyps/adenomas have this mutation (9,10). Oncogenic *BRAF* mutation induces proliferation of melanocytes and crypt epithelial cells and leads to formation of tumorous masses. However, most of them do not show continued proliferation or progress to malignant tumors. Nevi and hyperplastic crypts remain dormant for a long time with very low proliferative activity, elevated expression of $p16^{INK4a}$ and induction of senescence-associated acidic-β-galactosidase activity, representing OIS (8,11). In the genetically engineered mouse model, *BRAF* V600E mutation induces development of multiple lung adenomas. With continuous expression of *BRAF* V600E, the adenomas display initial high proliferative activity followed by growth arrest because of *BRAF*-induced

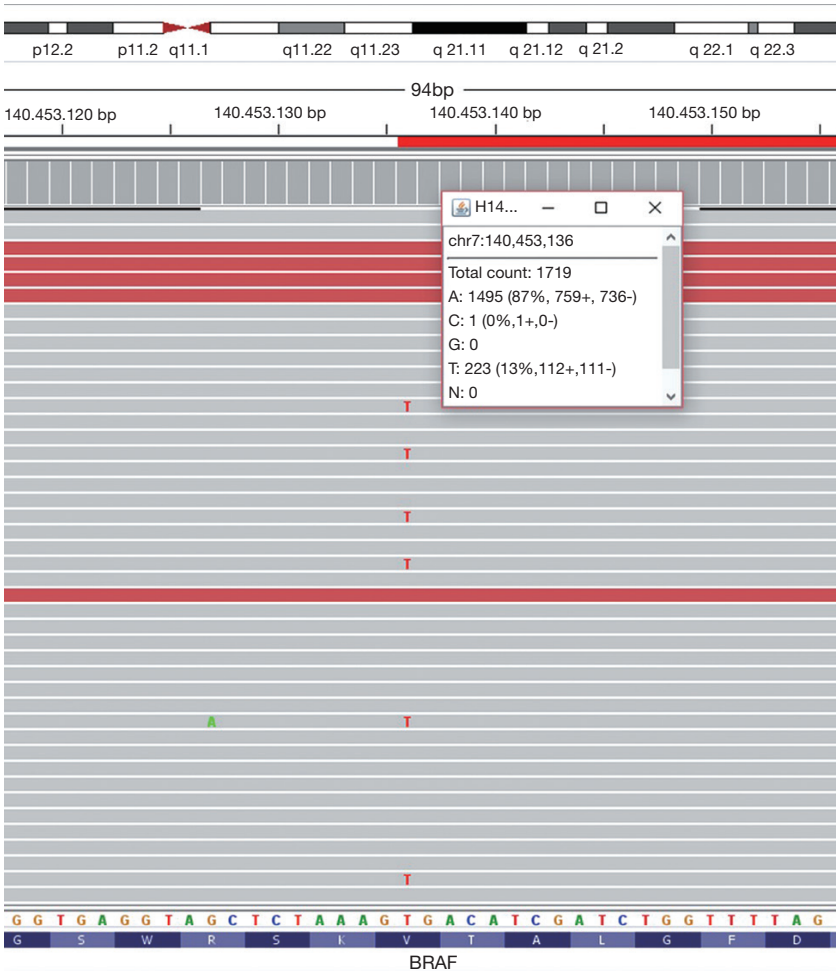

Figure 3 A *BRAF* V600E mutation detected with targeted deep sequencing. A substitution mutation in BRAF (c.1799A>T, p.V600E) was identified with mutant allele frequency of 13.0%.

senescence (12). Based on these data, we suggest that *BRAF* V600E is a driver mutation leading to tumorigenesis of CMPT and inducing OIS.

Although many types of malignant tumors show loss or epigenetic silencing of $p16^{INK4a}$, overexpression of $p16^{INK4a}$ may be maintained in some malignant tumors. The tumors caused by high-risk types of HPV show overexpression of $p16^{INK4a}$, or other retinoblastoma (RB) pathway dysregulation could lead to its activation (13). In these cases, coincidence of cellular proliferation is expected (7). Our case overexpressed $p16^{INK4a}$ without proliferative activity in addition to *BRAF* V600E mutation and the evidence of HPV infection was not identified. Therefore, we hypothesized that overexpression of $p16^{INK4a}$ accompanied by no proliferation in CMPT with *BRAF* V600E mutation might represent OIS.

Rare cases of CMPT harboring a component of well differentiated adenocarcinoma has been reported (14). *BRAF*-induced senescence is associated with the pathogenesis of some pilocytic astrocytoma and Langerhans cell histiocytosis and patients with tumors negative for $p16^{INK4a}$ show significantly shorter survival or aggressive behavior (15,16). Rare cases of acquired melanocytic nevi progress to malignant melanoma when they acquire additional mutations (8). It is assumed that, in cases of CMPT harboring *BRAF* mutations, failure to induce OIS or accumulation of additional mutations might be associated with malignant progression of this tumor.

Udo *et al.* reported a case of CMPT harboring *KRAS*

mutation, an oncogenic driver inducing OIS (5). However, the pathogenesis and clinical behavior might be different from our case because *KRAS*-induced OIS can be bypassed by abrogation of $p16^{INK4a}$-RB pathway (7). However, this is just an assumption based on the result of one case. The pathogenesis of CMPTs harboring other mutations or no mutation have not been elucidated yet. More cases with comprehensive molecular analysis need to be accumulated for supporting this hypothesis and establishing other pathogenetic mechanisms of CMPTs which have other driver mutations or no mutation.

According to the World Health Organization classification (17), pulmonary glandular papilloma is a benign endobronchial papillary tumor consisting of ciliated and nonciliated columnar cells and varying numbers of mucous cells. Most of these arise in the central lobar or segmental bronchi, while peripherally located pulmonary papillary tumors are very rare. Although their clinicopathologic features are not well characterized, pulmonary glandular papillomas and CMPTs have considerably overlapping histologic features, and the main differential may be endobronchial or non-endobronchial growth. It has not been determined whether they are different entities or representatives of a spectrum of the same disease. Recently, a case of mixed squamous cell and glandular papilloma showing overexpression of $p16^{INK4a}$ without evidence of HPV infection was reported (18). If driver oncogenes such as *BRAF* mutation are identified or $p16^{INK4a}$ overexpression is confirmed in pulmonary glandular papillomas, the hypothesis that two entities might form a spectrum of the same process with both ends of endobronchial and non-endobronchial tumors can be supportive.

In conclusion, we report a case of CMPT harboring *BRAF* V600E mutation, no proliferative activity and $p16^{INK4a}$ overexpression. CMPT is unique pulmonary neoplasm with characteristic pathological and molecular features. *BRAF* V600E mutation might be the driver oncogene inducing initiation of CMPT and leading to OIS, which represents indolent clinical behavior. Further comprehensive reviews with molecular studies are needed to define the nosological relationship of CMPT with pulmonary glandular papilloma.

Acknowledgements

Funding: This work was supported by Inha University Research Grant (No. 50469-01).

Footnote

Conflict of Interest: There authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Institutional Review Board of Inha University Hospital (IRB number: INHAUH 2016-08-015) and written informed consent was obtained from the patient for use of patient's tissue for molecular analysis and publication of this manuscript.

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Cite this article as: Kim L, Kim YS, Lee JS, Choi SJ, Park IS, Han JY, Kim JM, Chu YC. Ciliated muconodular papillary tumor of the lung harboring *BRAF* V600E mutation and $p16^{INK4a}$ overexpression without proliferative activity may represent an example of oncogene-induced senescence. J Thorac Dis 2017;9(12):E1039-E1044. doi: 10.21037/jtd.2017.11.120

