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Lung micropapillary adenocarcinomas revisited: A tale of antithesis with yearslong accumulative genetic alterations

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Henry Ford Hospital. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

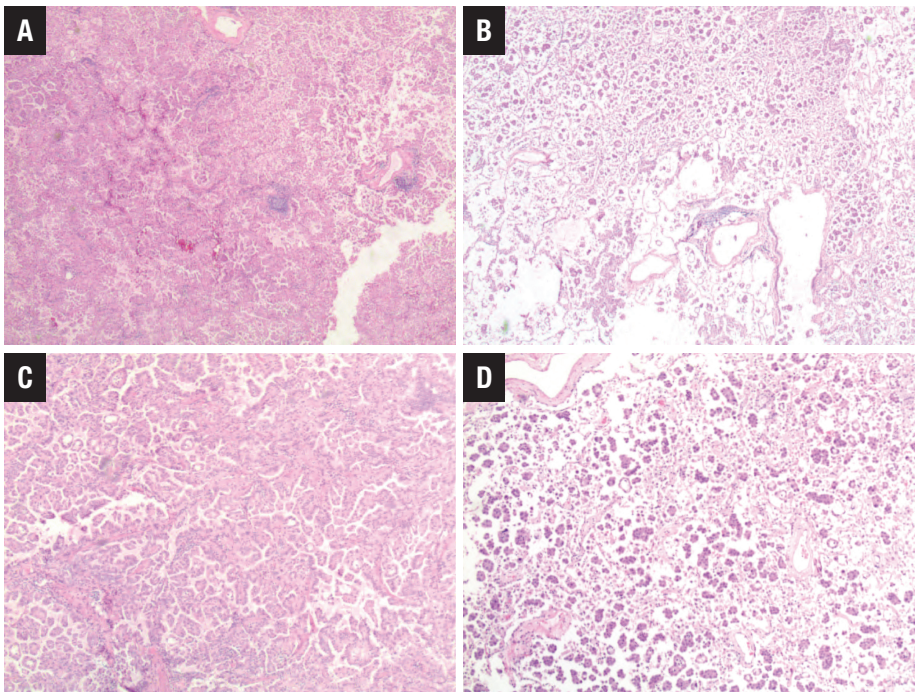
Ejas Palathingal Bava, MD
Zhiqiang B. Wang, MD, PhD

Micropapillary pattern of adenocarcinomas (MPC) are considered aggressive variants of lung adenocarcinomas. They have been associated with unfavorable outcome in terms of five-year survival in stage-matched patients with other morphological variants.[1] The presence of a micropapillary component of greater than five percent was reported as an independent risk factor for recurrence in patients treated with wedge resection or segmentectomy for lung adenocarcinomas 2 cm or smaller in size.[2] Other investigators stated that the prognosis is poor, regardless of the percentage of micropapillary component.[3] We herein report one case with a primary pT4 MPC that recurred 13 years after surgical resection. At the same time, we set out to delineate the molecular events and explore the pathophysiology behind the primary and recurrent tumors.

Case. The patient was a 78-year-old male with no history of smoking. He had a history of a left lower lobe lobectomy for a micropapillary predominant (>50 percent) lung adenocarcinoma 13 years earlier. The tumor measured 7.2 cm and was staged as pT4N0 (clinical stage IIIA). Other risk elements were negative, e.g. margin (1.5 cm to closest parenchymal margin), pleural invasion, and lymphovascular invasion (**Fig. 1A-B**). He received adjuvant chemotherapy with cisplatin/Navelbine after the primary tumor resection.

This time he presented with a biopsy-proven lung adenocarcinoma of the left upper lobe. PET imaging revealed negative mediastinal lymph nodes and distant metastasis. He underwent left upper lobe wedge resection that revealed 3.5-cm lung adenocarcinoma with morphologic similarity to the previously resected left lower lobe tumor (**Fig. 1C-D**). The tumor invaded visceral pleura and was 0.5 cm from the parenchymal margin. The sampled hilar lymph

Fig. 1. Representative H&E pictures of primary tumor (A, B) and recurrent tumor (C, D)



A) Primary tumor, center, mixed papillary and micropapillary pattern. **B)** Primary tumor, periphery, micropapillary pattern. **C)** Recurrent tumor, papillary pattern. **D)** Recurrent tumor, periphery, micropapillary pattern. All images were 20× magnified.

node was negative for metastasis. The tumor was staged as pT2aN0.

Next-generation sequencing using the Illumina MiSeq instrument and data analysis by the Sophia DDM platform with a panel of 53 genes were performed to determine and characterize the genetic alterations associated with the primary and recurrent tumors. Three NGS assays were performed on DNA extracted from formalin-fixed, paraffin-embedded tissue blocks: 1) microdissected MPC of the primary tumor, 2) microdissected papillary component (PC) of the primary tumor, and 3) recurrent tumor. RNA sequencing was not performed.

The NGS assays were monitored with the following benchmarks: 1) percent of targets with coverage greater than 500× (>95 percent), 2) coverage 10th quantile (>500), 3) coverage heterogeneity (<5 percent), 4) percent on target (enrichment) (>70 percent), and 5) duplication fraction (<90 percent). All of the NGS assays were successful, indicating high DNA quality after 13 years of preservation.

As summarized in **Table 1**, the primary lung adenocarcinoma demonstrated the following genetic alterations: two *TP53* tier one/two variants on the PC; two same *TP53* variants and a *MET* exon 14 skipping mutation on the MPC. Additionally, one tier three variant was also seen on the MPC portion. On the recurrent tumor, a total of 14 tier one/two variants were identified, including one same truncating *TP53* variant (*TP53* p.Arg213*) that was detected previously and one actionable *EGFR* variant, with an additional 88 tier three variants of unknown significance.

Discussion. In contrast to the notion that MPC is aggressive, our patient with stage III MPC lived 13 tumor-free years until recurrence. Behind the indolence were the morphologic features indicating that the peripherally abundant MPC appeared to have grown out of the centrally located papillary "nidus" as a secondary structure and the relative paucity of genetic alterations. Separate NGS assays performed on microdissected papillary component and MPC of the tumor detected two same *TP53* variants (c.853G>A, p.Gly285Lys; c.637C>T, p.Arg213*) in both portions. Such results were hence supportive of their clonal essence. *TP53* gene is the most common mutated gene in lung cancers.[4] In addition, the reduced allelic fractions of both *TP53* variants from 20 percent of PC to 10 percent of MPC implicated the subclonal rather than primary or de novo nature of MPC of this tumor. Subclonal evolution of the MPC was also supported by the detection of actionable *MET* c.3313+1G>A variant, which affects the evolutionally conserved splice junction leading to exon 14 skipping of the *MET* gene. Although it is debatable if the subclonal features of MPC obligated the tumor's clinical behavior similar to papillary adenocarcinoma, these unique findings should open research opportunities to improve our understanding.

While the discussion of clonal and subclonal tumor evolution highlighted the established notion of intratumoral genetic heterogeneity,[5] the recurrent tumor offered a rare chance to gain further insights. We noted that the same *TP53* variant (p.Arg213*) was detected in both NGS runs on the primary and recurrent tumors. According to our in-house data, the variant p.Arg213* of *TP53* was seen in one percent of all tumors tested. In addition to the same morphology, such a rare molecular event provided evidence supporting the clonal nature of the recurrent tumor.

Intriguingly, on top of this finding was the detection of numerous molecular events in the recurrent tumor. Such results were in sharp contrast to the primary tumor and highlighted the cumulative genetic damage over a 13-year period. It is noteworthy, however, that the patient's adjuvant chemotherapy after resection of the primary tumor may have had an impact on the genetic profiling of the recurrent tumor. It is known that chemotherapies are mutagenic and can contribute to tumor mutation burdens including inducing new mutations that render resistance to treatment, eliminating treatment-sensitive mutations, or driving tumor evolutionary patterns.[5] Hence, the genetic

Table 1. Molecular features of the primary and recurrent tumors using NGS

		TP53 (NM_000546.5)	*EGFR* (NM_005228.3)	*MET* (NM_001127500.2)	Other tier 1/2 variants/ VUS
Primary tumor	PC	c.853G>A, p.Gly285Lys (AF: 20%); c.637C>T, p.Arg213* (AF: 20%)			0/0
	MP	c.853G>A, p.Gly285Lys (AF: 11%); c.637C>T, p.Arg213* (AF: 10%)		c.3313+1G>A (AF: 5%)	0/1
Recurrent tumor		c.637C>T, p.Arg213* (AF: 40%); c.281C>A, p.Ser94* (AF: 18%)	c.2155G>T, p.Gly719Cys (AF: 6%)		11/88

PC and MP: papillary component and micropapillary component; AF: allelic fraction; NM_000546.5, NM_005228.3, NM_001127500.2: transcript IDs; VUS: variant of uncertain significance.

Tier designations by American College of Medical Genetics and Genomics (tiers 1–3 are reportable): tier 1: pathogenic; tier 2: likely pathogenic; tier 3: uncertain significance (VUS); tier 4: likely benign; tier 5: benign.

profile of the recurrent tumor should be interpreted as a combined result of biological acquisition during the long-term evolution and therapeutic induction.

Finally, it is worth mentioning that among genetic alterations in the recurrent tumor was the *EGFR* variant c.2155G>T, p.Gly719Cys. Along with the major *EGFR* gene exon 19 in frame deletions and exon 21 codon L858/L861 mutations, *EGFR* p.Gly719Cys has been identified as broadly sensitive to kinase inhibitors. The patient has been receiving osimertinib treatment and is doing well five years post-surgery.

Conclusion. We report a rare case of MPC lung adenocarcinoma that recurred in a different lung lobe after 13 years. We characterized the molecular events in different portions (PC and MPC) of the primary tumor and the recurrent tumor, with results demonstrating a relative paucity of genetic alterations in the primary tumor but strikingly numerous genetic alterations in the recurrent tumor. By telling this rare tale of antithesis to the established notion that MPC is aggressive, we explored the possible mechanisms behind the indolence and behind the differences in genetic alterations between the primary and recurrent tumors. □

1. Monroig-Bosque PDC, Morales-Rosado JA, Roden AC, et al. Micropapillary adenocarcinoma of lung: morphological criteria and diagnostic reproducibility among pulmonary pathologists. *Ann Diagn Pathol*. 2019;41:43–50.

2. Nitadori J, Bograd AJ, Kadota K, et al. Impact of micropapillary histologic subtype in selecting limited resection vs lobectomy for lung adenocarcinoma of 2cm or smaller. *J Natl Cancer Inst*. 2013;105(16):1212–1220.

3. Cao Y, Zhu LZ, Jiang MJ, Yuan Y. Clinical impacts of a micropapillary pattern in lung adenocarcinoma: a review. *Onco Targets Ther*. 2016;9:149–158.

4. Coleman WB, Tsongalis GJ, eds. *Essential Concepts in Molecular Pathology*. 2nd ed. Elsevier; 2019.

5. Venkatesan S, Swanton C, Taylor BS, Costello JF. Treatment-induced mutagenesis and selective pressures sculpt cancer evolution. *Cold Spring Harb Perspect Med*. 2017;7(8):a026617.

Dr. Palathingal Bava is a third-year pathology resident and Dr. Wang is senior staff pathologist, Department of Pathology and Laboratory Medicine, Henry Ford Hospital, Detroit.

Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports *and will be published next month in* CAP TODAY.

1. Which of the following is the most common mutated gene in lung cancers?
a. *KRAS*
b. *TP53*
c. *BRAF*
d. *EGFR*

2. Which of the following statements about lung adenocarcinomas is false?
a. Micropapillary pattern of adenocarcinomas (MPC) are not considered aggressive variants of lung adenocarcinomas.
b. The presence of a micropapillary component of greater than five percent is an independent risk factor for recurrence.
c. The adverse prognosis of MPC is directly proportional to the percentage of micropapillary component.
d. Patients with MPC have lower five-year survival in stage-matched patients compared with other morphological variants.

3. Which of the following statements about lung carcinomas is false?
a. MPC is not the result of expanded growth of the papillary component.
b. TTF-1 immunohistochemistry positivity is seen in lung adenocarcinomas.
c. The most frequent *EGFR* gene mutations are targetable for therapy.
d. Newer mutations are acquired during tumor evolution.