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Original contribution

Driver mutation profiles and clinicopathological correlation in pulmonary adenocarcinoma with a micropapillary component☆,☆☆

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Summary Pulmonary adenocarcinoma (PA) with a micropapillary component (PA-MPC) is considered a highly aggressive neoplasm. The molecular profile of PA-MPC has not yet been clearly elucidated. Based on these, we performed next-generation sequencing and quantitative real-time polymerase chain reaction (qPCR) to detect the driver mutation profiles of 50 PA-MPC cases and confirmed the results by Sanger sequencing. In addition, in 10 selected MPC-predominant cases, we captured the MPC and non-MPC by laser-capture microdissection and sequenced them separately to investigate the differences in driver mutation profiles between MPC and non-MPC. In 50 PA-MPC cases, the prevalence rates of *EGFR*, *KRAS*, and *PIK3CA* somatic mutations were 76.0%, 6.0%, and 2.0%, respectively; no *BRAF*, *NRAS*, *ALK*, *PDGFRA*, or other mutations were found. With regard to the MPC, *EGFR* mutation was more frequent in MPC-predominant cases (18/20; 90%) than in non–MPC-predominant cases (20/30; 66.7%). The overall survival of the MPC-predominant group was significantly worse than that of the non–MPC-predominant group. In the 10 microdissected MPC-predominant cases, the *EGFR* mutation was identical in both components and was consistent with previous results without microdissection. In conclusion, our study indicated that *EGFR* mutations were frequent in PA-MPC. Paired MPC and non-MPC from the same cases had the same driver mutation profiles.

1. Introduction

Lung cancer is the main cause of cancer-related death worldwide [1,2], and pulmonary adenocarcinoma (PA) is the most common histologic subtype, accounting for almost half

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of lung cancers [3]. In the international, multidisciplinary classification of lung adenocarcinoma, which was adopted by the World Health Organization (WHO) in 2015 [4,5], PA with a micropapillary component (PA-MPC) was recommended as a new subtype of PA that differs from other subtypes, including lepidic, acinar, papillary, and solid, as defined in the 2004 WHO classification [5,6].

The histologic characteristics of micropapillary component (MPC) were first described by Amin et al [7]. Since then, the histology of tiny papillary tufts lying in alveolar spaces or loose connective tissues has been named MPC, and several studies on PA-MPC have been reported. Some results have suggested that MPC may be a prognostic predictor of a poor outcome [7-12]. Although PA-MPC has been defined as a highly aggressive tumor, its molecular profiles have yet to be clearly elucidated.

Intratumoral diversity in several tumors, including PA, is an obstacle to understanding the precise molecular profiles of tumors [13-16]. In view of this finding, laser-capture microdissection (LCM) and tissue subtype capture are suitable methods to avoid the heterogeneity of intratumoral diversity and accurately assess the molecular characteristics of tumor components [14].

Driver genes, also known as functional genes, are the type of gene that exerts a functional contribution in multiple cancers. It is vital to identify the alterations of driver genes in tumor initiation and progression. We aimed to detect the frequency of common driver oncogene alterations in PA-MPC and analyze intratumoral diversity.

2. Materials and methods

2.1. Case selection

Overall, MPC was defined by small papillary tufts lacking a central fibrovascular core lying freely within alveolar spaces or in the clefts or space of fibrous tissue (Fig. 1A) [7-12]. Fifty surgical resected cases of PA-MPC at the Peking Union Medical College Hospital between January 2010 and December 2011 that contained more than 10% MPC were selected for the detection of common driver mutations. In addition, 10 MPC-predominant cases with distinct areas of diverse components were further investigated to identify differences of in MPC (Fig. 1A) and non-MPC (Fig. 1B) by LCM. Pathological characteristics were obtained from reviewing the slides in detail. The histologic types of the tumor were described according to the WHO classification system of 2015 [4].

Clinical information on PA-MPC patients was collected from the clinical archives. Progression-free survival (PFS) was defined as the time from surgery to relapse or the end point of the study, and overall survival (OS) was defined as the time from surgery to death or the study termination. TNM staging was performed according to the classification of the Union Internationale Contre le Cancer [17]. This study was conducted with the approval of the Ethics Committee of Peking Union Medical College Hospital, and informed consent was obtained from all patients.

2.2. Macrodissection or microdissection and genomic DNA extraction

Sections (4 μm thick) were cut from formalin-fixed, paraffin-embedded (FFPE) tissue blocks, and 10 consecutive sections of each block were acquired. Nine sections were manually macrodissected according to hematoxylin and eosin staining, from which the MPC portion was scraped off into a microfuge tube to obtain tumor DNA.

In 10 selected MPC-predominant cases, the different driver mutation profiles in MPC and non-MPC were further investigated in 10-μm-thick sections of each FFPE specimen collected on polyethylene naphthalate membrane slides (Leica Microsystem, Wetzlar, Germany) designed specifically for microdissection. After deparaffinization, each tissue slide was stained with hematoxylin, and different cancer components were confirmed independently by 2 experienced pathologists. MPC and non-MPC were separately microdissected with LCM using a Leica LMD6500 system (Leica Microsystems; Fig. 1C-E).

Genomic DNA was isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). DNA concentration was measured with NanoDrop Spectrometer 2000 (ThermoFisher, Wilmington, DE) and normalized to 20 to 50 ng/μL. DNA samples were stored at −20°C before utilization.

2.3. Genetic mutation detection by next-generation sequencing

Mutation analysis was performed by next-generation sequencing (NGS). The NextDaySeq-lung Cancer Library Preparation Panel Kit (Beijing ACCB Biotech, Beijing, China) for the Ion Torrent System (Life Technologies, Wilmington, DE) was used to construct libraries. Briefly, targeted genomic regions (Supplementary Table 1) were amplified using pooled primer pairs. After adaptor and barcode ligation and purification, the libraries were quantified and diluted to a concentration of 3 ng/mL, after pooling. The library pool was sequenced using Ion Torrent PGM system. A proprietary bioinformatics pipeline, the DanPA bioinformatics pipeline, was used for variant calling and annotation. The pipeline was specifically designed for the NextDaySeq panels. The cutoff of mutation frequency for mutation calling was 1%, and that of mutation reads was 5.

2.4. Quantitative real-time PCR

Mutation profiles for *EGFR*, *KRAS*, and *PIK3CA* were examined using the Human *EGFR* Gene Mutation Detection Kit (Beijing ACCB Biotech, Beijing, China), Human *KRAS* Gene Mutation Detection Kit (Beijing ACCB Biotech, Beijing, China) and Human *PIK3CA* Gene Mutation Detection Kit (Beijing ACCB Biotech, Beijing, China), respectively. The

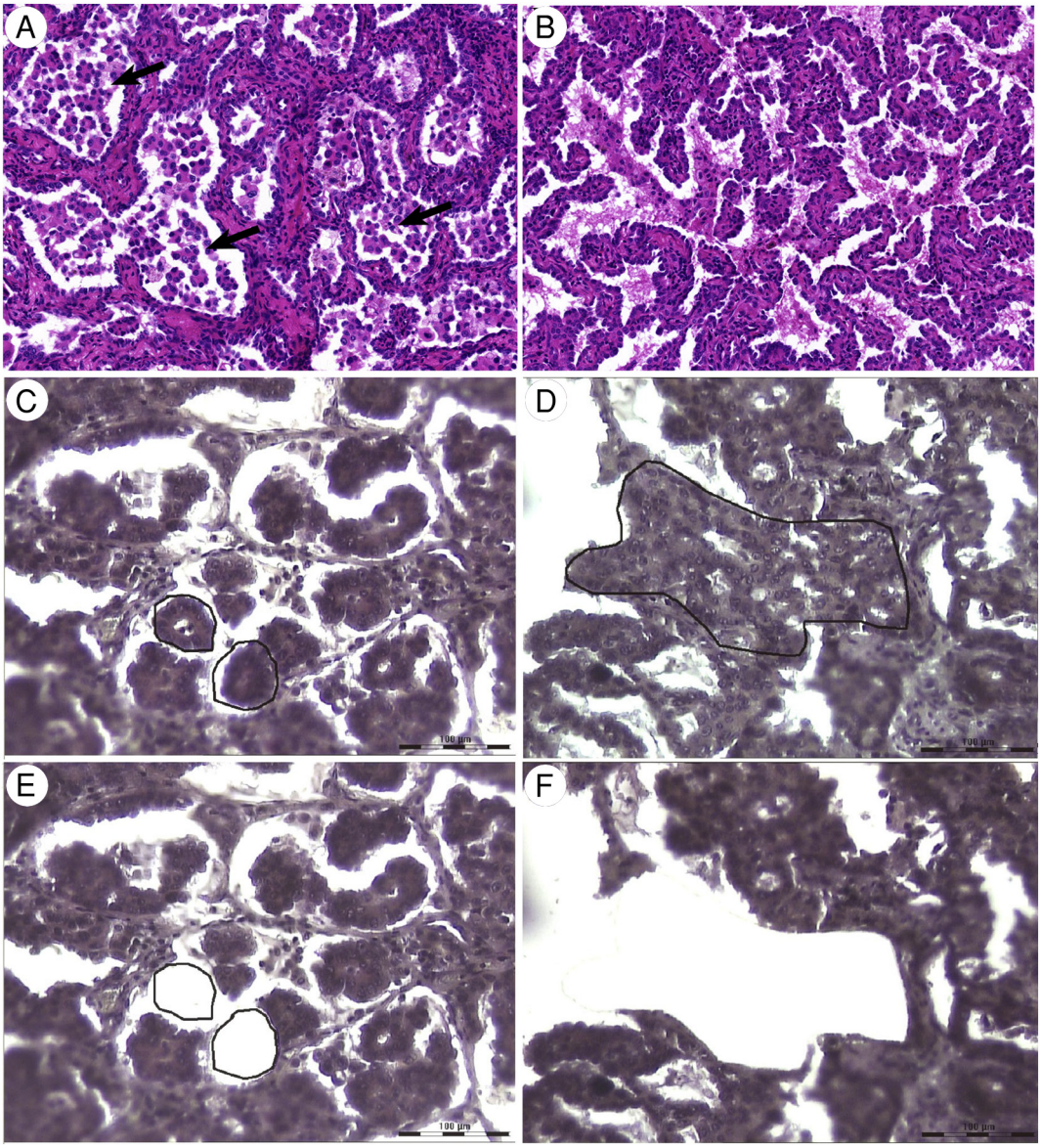

Fig. 1 E;Hematoxylin and eosin staining showed the MPC (A) and the non-MPC (B). The MPC was characterized by small papillary tufts lacking a central fibrovascular core lying freely within alveolar spaces (arrows; magnification ×20). C and D, The hematoxylin staining showed the MPC and non-MPC under Leica LMD6500 system. The MPC and non-MPC could be verified under microscope (the outlines in panels C and D, respectively) and microdissected by LCM (E and F; magnification ×20).

tests covered 62 hotspot mutations including 45 in exons 18, 19, 20, and 21 of *EGFR*, 12 in exons 2 and 3 of *KRAS* and 5 in exons 9 and 20 of *PIK3CA*. qPCR was performed using an Mx3000P PCR instrument (Agilent, Santa Clara, CA) with the following settings: 95°C for 10 minutes, and 40 cycles of 95°C for 15 s and 60°C for 1 minute. The results were interpreted according to the manufacturer's instructions.

2.5. Sanger sequencing

The Sanger sequencing method was used in this study for result confirmation. Genomic regions of *EGFR* exons 18 to 21, *KRAS* exons 2 to 3, and *PIK3CA* exons 9 and 20 were amplified using DNA samples. Each exon was sequenced bidirectionally using the primers that were used for the initial amplification reaction and the ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequencing primer extension reactions were analyzed using the ABI 3130XL Genetic Analyzer (Applied Biosystems).

2.6. Statistical analyses

Patient characteristics were summarized as descriptive statistics. Associations among genetic mutations and clinicopathological factors were evaluated by the χ^2 test or, where appropriate, Fisher exact test. The mean values of continuous

data were assessed using independent-samples *t* test. Survival curves were plotted according to the Kaplan-Meier analysis, and multivariate analysis according to the Cox regression model was performed. The data were analyzed with SPSS software (version 20.0; Chicago, IL). *P* values less than .05 were considered significant.

3. Results

3.1. Clinicopathological characteristics

A total of 50 PA-MPC cases with MPC >10% were included in this study, including 28 men and 22 women, and 17 smokers and 33 nonsmokers were verified. The age of the patients ranged from 37 to 81 years, with a mean of 58.5 years. The average size of tumor was 3.6 cm (range, 1.5-9.0 cm). According to TNM staging, 24 patients (48%) were classified as stage I, 3 patients (6%) as stage II, 21 patients (42%) as stage III, and 2 patients (4%) as stage IV (Table). Adequate follow-up information was obtained for 49 patients. The follow-up period ranged from 7 to 78 months, with a mean of 33.7 months. Recurrence or metastasis was evaluated by radiologic investigations. In this study, 17 patients (34%) experienced recurrence or metastasis after an average of 23.3 months (range, 10-35 months). Six patients (12%) died of their cancer after surgery (range, 7-38 months), and 27 patients were alive without evidence of disease.

According to the WHO classification system in 2015, the cases were classified either as the MPC-predominant group (20/50 [40%]) or the non-MPC-predominant group (30/50 [60%]), including lepidic predominant (2/50 [4%]), acinar predominant (12/50 [24%]), papillary predominant (14/50 [28%]), and solid predominant (2/50 [4%]). Twenty-one patients had lymphovascular invasion (42%), whereas 26 had lymph node metastasis (52%). In addition, bronchus involvement was identified in 28 cases (56%) and pleural invasion in 20 cases (40%).

Survival curves depicted by Kaplan-Meier showed that OS of the MPC group was significantly shorter than that of the non-MPC group ($P = .036$; Fig. 2).

3.2. Comparison of NGS, qPCR, and Sanger sequencing results

Sufficient DNA of 42 cases was obtained for NGS analysis, whereas all 50 samples of DNA were successfully detected by

Table Relationship between *EGFR* and *KRAS* mutations and the patients' clinical characteristics in PA-MPCs

Characteristics	Total	*EGFR* mutation			*KRAS* mutation		
		Positive	Negative	*P*	Positive	Negative	*P*
No. of cases	50	38 (76.0%)	12 (24.0%)		3 (6.0%)	47 (94.0%)	
Age (y), mean ± SD	58.5 (37-81)	58.5 ± 9.9	63.8 ± 9.4	.109	67.3 ± 7.6	59.3 ± 10.0	.180
Sex							
Male	28 (56.0%)	18	10	.029 *	3	25	.113
Female	22 (44.0%)	20	2		0	22	
Smoking status							
Nonsmoker	33 (66.0%)	28	5	.041 *	1	32	.218
Smoker	17 (34.0%)	10	7		2	15	
Tumor size (cm), mean ± SD	3.6 (1.5-9.0)	3.1 ± 1.1	5.2 ± 2.1	<.001 *	6.8 ± 2.6	3.4 ± 1.3	<.001 *
Lymph node metastasis							
Negative	24 (48.0%)	17	7	.411	2	22	.504
Positive	26 (52.0%)	21	5		1	25	
Pleural invasion							
Negative	30 (60.0%)	23	7	.892	1	29	.331
Positive	20 (40.0%)	15	5		2	18	
Lymphatic and venous invasion							
Negative	29 (58.0%)	22	7	.979	2	27	.754
Positive	21 (42.0%)	16	5		1	20	
Bronchus invasion							
Negative	22 (44.0%)	16	6	.631	1	21	.701
Positive	28 (56.0%)	22	6		2	26	
Histologic subtype							
MPC-predominant	20 (40.0%)	18	2	.058	1	19	.808
Non–MPC-predominant	30 (60.0%)	20	10		2	28	
TNM stage							
I-II	27 (52.0%)	20	7	.730	2	25	.650
III-IV	23 (46.0%)	18	5		1	22	

* *P* is statistically significant.

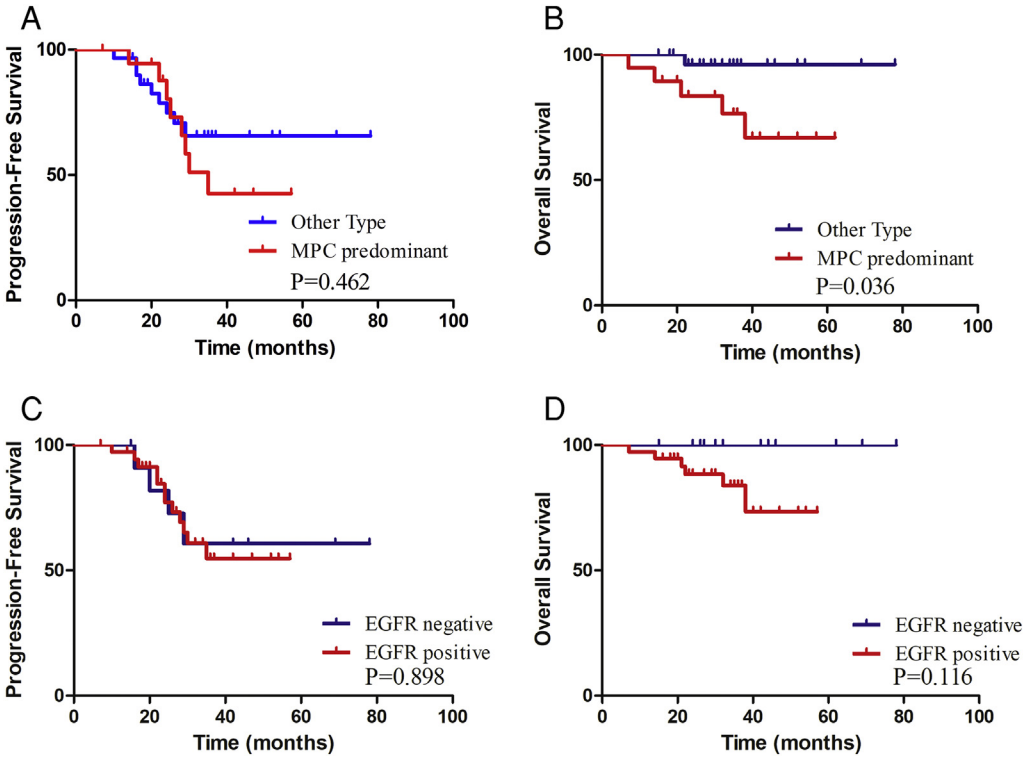

Fig. 2 Kaplan-Meier survival curves. A and B, PFS and OS of patients in the groups with different histologic subtypes. C and D, PFS and OS of patients in the groups with different *EGFR* mutations.

qPCR. Detailed nucleotide alterations detected by qPCR and NGS are listed in the Supplementary Table. Most cases showed consistent results; inconsistencies between qPCR and NGS were observed in 2 cases. One inconsistent case showed *EGFR* exon 21 mutation detected by NGS, whereas qPCR detected no mutations. Another case showed *EGFR* exon 19 deletion detected by qPCR but was negative for mutation by NGS. To validate the *EGFR* status, these 2 cases were examined by Sanger sequencing, and *EGFR* mutations were observed in the 2 cases.

3.3. Genetic alteration profiles

The overall mutation frequency was 82.0% (41/50) in all PA-MPCs. Notably, *EGFR* is the major mutated gene in PA-MPCs with a prevalence rate of 76.0% (38/50). The prevalence of *KRAS* and *PIK3CA* somatic mutations was 6.0% (3/50) and 2.0% (1/50; which coexisted with *EGFR* mutation), respectively (Fig. 3), and no somatic mutations were detected in *BRAF*, *NRAS*, *ALK*, *PDGFRA*, or other genes.

The most frequent *EGFR* mutation was an in-frame deletion in exon 19 (18/50 [36%]; Fig. 4A), followed by a point mutation in exon 21 (15/50 [30%]), resulting in substitution of leucine by arginine at codon 858 (Fig. 4B) and leucine by glutamine at codon 861 (L858R, 14/50 [28%]; L861Q, 1/50 [2%]). A drug-resistant exon 20 insertion mutation was observed in only 1 case. Combined mutations in exon 20 (S768I) and exon 18 (G719A) were detected in 2 cases. G719A point mutation was observed in 2 cases.

KRAS mutations were identified in 3 (6.0%) of the 50 PA-MPC patients. All 3 cases had single amino acid substitutions

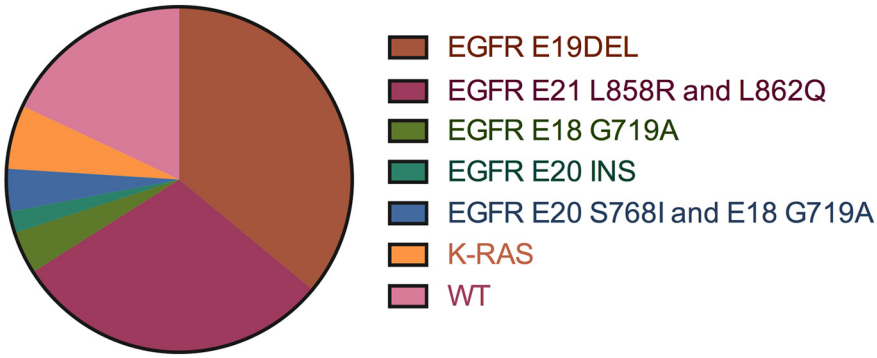

Fig. 3 Frequency of major driver mutations in PA-MPC.

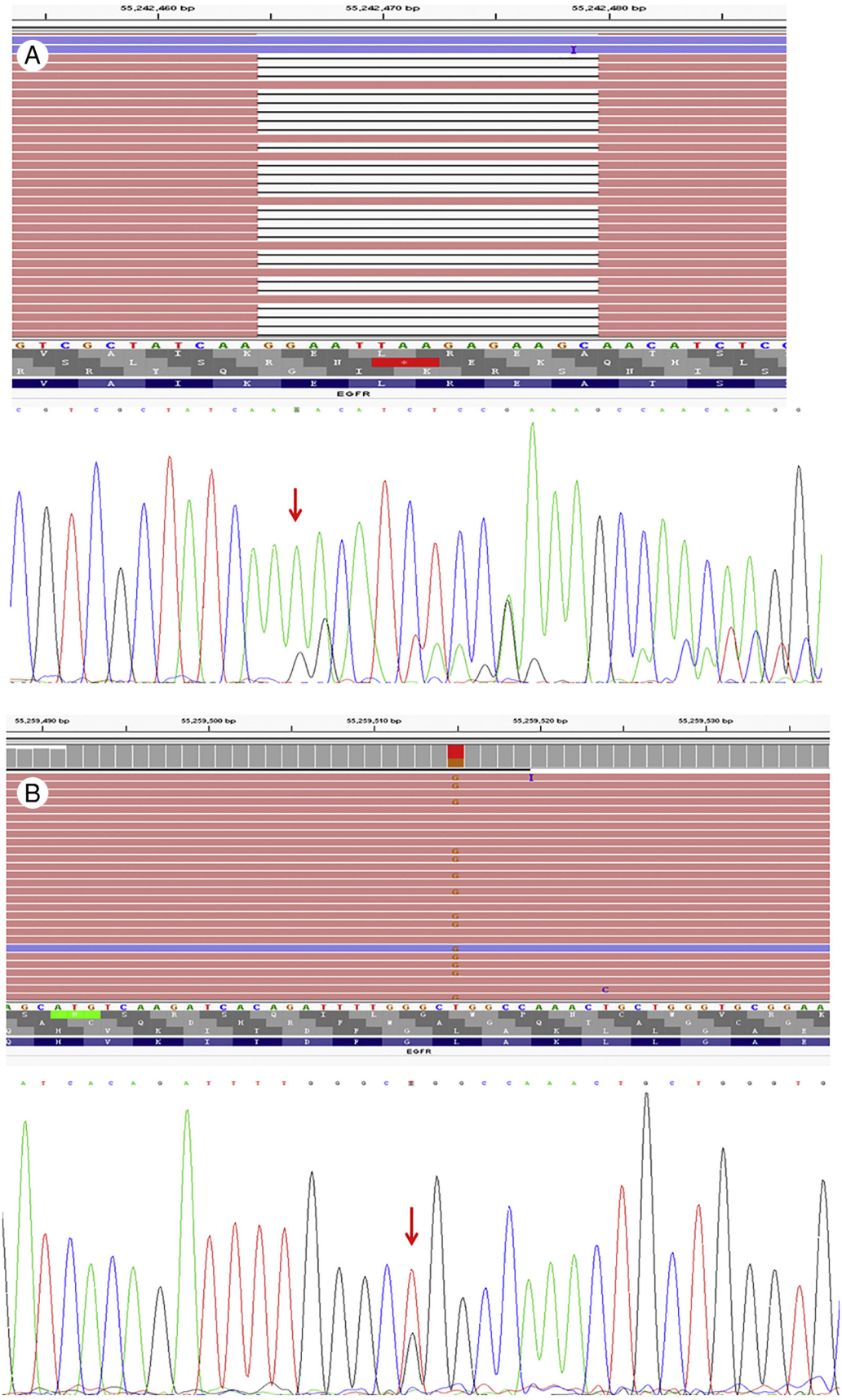

Fig. 4 A, *EGFR* exon 19 deletions in 1 case. NGS chromatogram and Sanger sequencing chromatogram. B, *EGFR* exon 21 L858R mutations in 1 case. NGS chromatogram and Sanger sequencing chromatogram.

in codon 12, including 1 in 12Cys, 1 in 12Val, and 1 in 12Asp. No tumor simultaneously harbored both *EGFR* and *KRAS* gene mutations. *PIK3CA* mutation was detected in only 1 case (2% [1/50]), which coexisted with *EGFR* mutation.

For the 10 MPC-predominant cases in which the MPC and non-MPC were microdissected and sequenced separately, the results of NGS showed that the status of *EGFR* mutation was identical in both components, and the mutation types were consistent with previous samples analyzed without LCM. In addition, *KRAS*, *PIK3CA*, or any other gene mutations were absent.

3.4. Correlation between genetic alterations and clinicopathological findings

The associations between *EGFR* or *KRAS* and clinicopathological factors are summarized in Table. *EGFR* mutations were significantly associated with smoking status ($P = .041$), sex ($P = .029$), and tumor size ($P < .001$), but no obvious correlation between *EGFR* mutations and age ($P = .109$), lymph node metastasis ($P = .411$), pleural invasion ($P = .892$), lymphatic and venous invasion ($P = .979$), or clinical stage ($P = .730$). *EGFR* mutation was detected in 2 lepidic predominant cases (2/2 [100%]), 8 acinar predominant cases (8/12 [66.7%]), and 10 papillary predominant cases (10/14 [71.4%]) but no solid predominant cases (0/2 [0%]). For the MPC, *EGFR* mutation was more frequent in the MPC-predominant group (18/20 [90%]) than in the non–MPC-predominant group (20/30 [66.7%]; $P = .058$), without reaching significance.

KRAS occurred more frequently in larger tumors ($P < .001$). No significant associations were observed between the groups positive and negative for *KRAS* mutations with respect to other clinicopathological factors. However, no obvious association between *EGFR*, *KRAS* mutation and prognosis of PA-MPC was observed (Fig. 2).

4. Discussion

The International Association for the Study of Lung Cancer/the American Thoracic Society/the European Respiratory Society classification based on a multidisciplinary teamwork adds a new MPC-predominant subtype in lung adenocarcinoma [5]. Almost all of the literature on PA-MPC has indicated that it is a highly invasive subtype with a poor prognosis [7-12].

In our study, we detected the frequency of common driver oncogene alterations in the PA-MPC using NGS and qPCR. We discovered a prevalence of 76% somatic mutations in *EGFR* in PA-MPC. To date, only 3 articles focusing on *EGFR* mutation status in PA-MPC have been reported after the new classification was proposed. Chao et al [18] reviewed 79 cases of MPC-positive tumors (MPC ratio ≥5%) and found that *EGFR*-mutated tumors were often MPC positive ($P < .001$), which suggests an association between *EGFR* mutation and MPC. Cai et al [19] showed that *EGFR* mutation was significantly more frequent in adenocarcinomas with papillary (85.7%) or MPC (91.4%; $P < .001$), whereas Furukawa et al [20] showed no association between *EGFR* mutations and MPC ($P > .05$). In our study, we found that *EGFR* mutations were the most frequent gene alteration in PA-MPC, with a higher mutation frequency (76%) than other types of PA that exhibited *EGFR* mutations at rate of 30% to 63.9% [19,21-23]. Furthermore, *EGFR* mutation was found to be significantly more common in MPC-predominant adenocarcinomas than other subtype predominant adenocarcinomas in our study, which revealed that *EGFR* mutations could be associated with MPC-predominant adenocarcinomas.

Previous large cohort studies have shown that *EGFR* mutation was different in diverse predominant subtypes of PA [24]. Yoshizawa et al [25] showed that *EGFR* mutation was observed in 90 patients (53.9%) and demonstrated a significant association between *EGFR* mutation and PA with nonmucinous lepidic and papillary components. *EGFR* mutation was identified more frequently in the MPC-predominant ($P = .0068$) and lepidic component ($P = .005$) subtypes [26]. Cai et al also demonstrated that *EGFR* mutation was associated with sex, smoking history, and histologic characteristics. Similarly, our results suggested that PA with *EGFR* mutations could be observed more frequently in female nonsmokers. In addition, we found that the tumor size was significantly associated with positive *EGFR* mutation ($P < .001$). The 2 major types of *EGFR* mutation in this study were in-frame deletions in exon 19 (18/50) and exon 21 L858R (14/50), which accounted for more than 90% of the detected mutations. We also identified 1 case of exon 20 insertion mutation, which is related to tyrosine kinase inhibitor resistance [24]. No significant difference was observed in clinical outcome between patients with wild-type *EGFR* and those with mutant *EGFR* ($P = .968$). Some studies found that patients with *EGFR* mutations had prolonged PFS ($P = .011$) and OS ($P = .046$) compared with patients negative for *EGFR* mutation, whereas other studies showed no association [27,28]. Perhaps the diverse results in different studies may reflect different follow-up times and individual responses to radiotherapy and chemotherapy.

Similar to *EGFR* mutation, some studies have shown that *KRAS* mutation is also associated with worse clinical outcomes and tyrosine kinase inhibitor resistance [29]. In our study, *KRAS* mutation was detected in 3 of the 50 patients and exclusively to *EGFR* mutations [12], which was consistent with our previous study. Our findings suggested that *KRAS* mutations might be present at a low frequency in Chinese PA-MPC patients. Studies from Eastern Asia reported fewer *KRAS* mutations in PA-MPC compared with European and American studies [30,31]. Because the molecular profiles differed from different studies, it may be in a way attributed to the ethnicity or geographical distribution as well as the use of sensitive techniques in different studies. Furukawa et al [20] used SNaPshot assay based on multiplex polymerase chain reaction method as a highly sensitive test assay, whereas De Oliveira Duarte Achcar et al [32] performed the study using a less sensitive direct DNA sequencing method. In this study, we chose qPCR and NGS to detect mutations in all of 50 cases. Because

9 cases with low DNA library concentrations did not reach the requirements of NGS, we used qPCR to detect all 50 cases and NGS for 41 cases. In the cases analyzed by the 2 methods, most results were concordant, whereas only 2 results were inconsistent. Sanger sequencing was selected to provide a final confirmation of the results. Overall, we detected genetic alterations by 3 methods to identify the somatic mutation in PA-MPC and detected a higher prevalence of *EGFR*.

As intratumoral diversity was discovered in several tumors, including PA [14], which should be considered when analyzing genetic mutations. To minimize the bias of intratumoral diversity, LCM was performed to acquire enriched MPC. We captured MPC under a microscope after precise laser dissection. Cai et al [19] performed LCM to acquire DNA of pure MPC constituent and detected the molecular profiles of PA-MPC by amplification refractory mutation system and found 3 types of *EGFR* mutation: deletion in exon 19, L858R or T790M, and G719X or L861Q. We did not detect *EGFR* T790M in our specimens, and we believe that this inconsistency is because Cai et al selected cases with drug resistance, whereas drug resistance was not included in our criteria. We intend to further study the mechanism between *EGFR* T790M mutation and drug resistance in PA.

In conclusion, we plotted the driver mutation profiles of PA-MPC in the present study and revealed the clinicopathological correlation. Our research indicated that *EGFR* mutations were frequent in PA-MPC, and both MPC and non-MPC in the same case have the same driver mutation profiles.

Author contributions

J. Z. designed the study and interpreted the data. J. S. and A. W. contributed to the selection and collection of specimens. Z. Z. performed the statistical analysis and wrote the manuscript. J. S. and X. L. performed the microdissections and macrodissections. J. L. prepared the figures and tables. Z. L. supervised the project. All authors fulfilled the International Committee of Medical Journal Editors criteria and approved the final manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.11.008.

References

[1] Chen W, Zheng R, Zeng H, Zhang S. Epidemiology of lung cancer in China. Thorac Cancer 2015;6:209-15. https://doi.org/10.1111/1759-7714.12169.

[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5-29. https://doi.org/10.3322/caac.21254.

[3] Curado MP, Edwards B, Shin HR, Storm H, Ferlay J. Cancer Incidence in Five Continents, vol. IX. Lyon: IARC Scientific Publications; 2007.

[4] Travis WD, Brambilla E, Burke AP, Müller-Hermelink HK, Harris CC. World Health Organization classification of tumours of the lung, pleura, thymus and heart. In: Bosman FT, Jaffe ES, Lakhani SR, Ohgaki H, editors. World Health Organization Classification of Tumors. Lyon: IARC Press; 2015.

[5] Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244-85. https://doi.org/10.1097/JTO.0b013e318206a221.

[6] Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC, Burke AP. World Health Organization Classification of Tumors. Pathology and Genetics Tumors of the Lung, Plrura, Thymus and Heart. Lyon: IARC Press; 2004.

[7] Amin MB, Tamboli P, Merchant SH, et al. Micropapillary component in lung adenocarcinoma: a distinctive histologic feature with possible prognostic significance. Am J Surg Pathol 2002;26:358-64.

[8] Kamiya K, Hayashi Y, Douguchi J, et al. Histopathological features and prognostic significance of the micropapillary pattern in lung adenocarcinoma. Mod Pathol 2008;21:992-1001. https://doi.org/10.1038/modpathol.2008.79.

[9] Miyoshi T, Satoh Y, Okumura S, et al. Early-stage lung adenocarcinomas with a micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis. Am J Surg Pathol 2003;27:101-9.

[10] Sanchez-Mora N, Presmanes MC, Monroy V, et al. Micropapillary lung adenocarcinoma: a distinctive histologic subtype with prognostic significance. Case series. Hum Pathol 2008;39:324-30. https://doi.org/10.1016/j.humpath.2007.05.029.

[11] Tsutsumida H, Nomoto M, Goto M, et al. A micropapillary pattern is predictive of a poor prognosis in lung adenocarcinoma, and reduced surfactant apoprotein A expression in the micropapillary pattern is an excellent indicator of a poor prognosis. Mod Pathol 2007;20:638-47. https://doi.org/10.1038/modpathol.3800780.

[12] Zhang J, Liang Z, Gao J, Luo Y, Liu T. Pulmonary adenocarcinoma with a micropapillary pattern: a clinicopathological, immunophenotypic and molecular analysis. Histopathology 2011;59:1204-14. https://doi.org/10.1111/j.1365-2559.2011.04050.x.

[13] Crouch EC, Stone KR, Bloch M, McDivitt RW. Heterogeneity in the production of collagens and fibronectin by morphologically distinct clones of a human tumor cell line: evidence for intratumoral diversity in matrix protein biosynthesis. Cancer Res 1987;47:6086-92.

[14] Ma P, Fu Y, Cai MC, et al. Simultaneous evolutionary expansion and constraint of genomic heterogeneity in multifocal lung cancer. Nat Commun 2017;8:823. https://doi.org/10.1038/s41467-017-00963-0.

[15] Merlo LM, Maley CC. The role of genetic diversity in cancer. J Clin Invest 2010;120:401-3. https://doi.org/10.1172/JCI42088.

[16] Wei L, Wang J, Lampert E, et al. Intratumoral and intertumoral genomic heterogeneity of multifocal localized prostate cancer impacts molecular classifications and genomic prognosticators. Eur Urol 2017;71:183-92. https://doi.org/10.1016/j.eururo.2016.07.008.

[17] Sobin LH, Wittenkind CH. TNM Classification of Malignant Tumours. 6th ed. New York: Wiley-Liss; 2002.

[18] Chao L, Yi-Sheng H, Yu C, et al. Relevance of EGFR mutation with micropapillary pattern according to the novel IASLC/ATS/ERS lung adenocarcinoma classification and correlation with prognosis in Chinese patients. Lung Cancer 2014;86:164-9. https://doi.org/10.1016/j.lungcan.2014.08.018.

[19] Cai YR, Dong YJ, Wu HB, et al. Micropapillary: a component more likely to harbour heterogeneous EGFR mutations in lung adenocarcinomas. Sci Rep 2016;6:23755. https://doi.org/10.1038/srep23755.

[20] Furukawa M, Toyooka S, Ichimura K, et al. Genetic alterations in lung adenocarcinoma with a micropapillary component. Mol Clin Oncol 2016;4:195-200. https://doi.org/10.3892/mco.2015.690.

[21] Clay TD, Russell PA, Do H, et al. Associations between the IASLC/ATS/ERS lung adenocarcinoma classification and EGFR and KRAS mutations. Pathology 2016;48:17-24. https://doi.org/10.1016/j.pathol.2015.11.002.

[22] Li S, Choi YL, Gong Z, et al. Comprehensive characterization of oncogenic drivers in asian lung adenocarcinoma. J Thorac Oncol 2016;11:2129-40. https://doi.org/10.1016/j.jtho.2016.08.142.

[23] Liang Z, Zhang J, Zeng X, Gao J, Wu S, Liu T. Relationship between EGFR expression, copy number and mutation in lung adenocarcinomas. BMC Cancer 2010;10:376. https://doi.org/10.1186/1471-2407-10-376.

[24] Woo HS, Ahn HK, Lee HY, et al. Epidermal growth factor receptor (EGFR) exon 20 mutations in non–small-cell lung cancer and resistance to EGFR–tyrosine kinase inhibitors. Invest New Drugs 2014;32:1311-5. https://doi.org/10.1007/s10637-014-0146-x.

[25] Yoshizawa A, Sumiyoshi S, Sonobe M, et al. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol 2013;8:52-61. https://doi.org/10.1097/JTO.0b013e3182769aa8.

[26] Song Z, Zhu H, Guo Z, Wu W, Sun W, Zhang Y. Correlation of EGFR mutation and predominant histologic subtype according to the new lung adenocarcinoma classification in Chinese patients. Med Oncol 2013;30:645. https://doi.org/10.1007/s12032-013-0645-1.

[27] Lim KH, Huang MJ, Liu HC, Kuo HT, Tzen CY, Hsieh RK. Lack of prognostic value of EGFR mutations in primary resected non–small cell lung cancer. Med Oncol 2007;24:388-93.

[28] Liu WS, Zhao LJ, Pang QS, Yuan ZY, Li B, Wang P. Prognostic value of epidermal growth factor receptor mutations in resected lung adenocarcinomas. Med Oncol 2014;31:771. https://doi.org/10.1007/s12032-013-0771-9.

[29] Karachaliou N, Mayo C, Costa C, et al. KRAS mutations in lung cancer. Clin Lung Cancer 2013;14:205-14. https://doi.org/10.1016/j.cllc.2012.09.007.

[30] Riely GJ, Marks J, Pao W. KRAS mutations in non–small cell lung cancer. Proc Am Thorac Soc 2009;6:201-5. https://doi.org/10.1513/pats.200809-107LC.

[31] Wang Z, Wu YL, Zhang GC, Zhou Q, Xu CR, Guo AL. EGFR/KRAS mutations and gefitinib therapy in Chinese NSCLC patients. Onkologie 2008;31:174-8. https://doi.org/10.1159/000116736.

[32] De Oliveira Duarte Achcar R, Nikiforova MN, Yousem SA. Micropapillary lung adenocarcinoma: EGFR, K-ras, and BRAF mutational profile. Am J Clin Pathol 2009;131:694-700. https://doi.org/10.1309/AJCPBS85VJEOBPDO.