TERT Mutation was Associated with the Risk of Recurrence in Lung Adenocarcinoma with A Micropapillary Pattern

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Research article

Keywords: lung adenocarcinoma with a micropapillary pattern, next-generation sequencing, mutation profiling, prognosis

Posted Date: July 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-39789/v1>

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Abstract

Background: Lung adenocarcinoma with a micropapillary pattern (MPPAC) is the histological subtype of lung cancer. It has attracted increasing attention, especially regarding its association with poor prognosis, including the predisposition towards recurrence and metastasis. Although MPPAC has been described in early-stage cases, only a few studies have reported the correlation between disease-specific prognosis and gene mutation of MPPAC. This study aimed to clarify the common genetic mutations and the prognostic characteristics in MPPAC patients.

Methods: A total of 17 patients whose surgical pathology was defined as MPPAC were followed up, the molecular characteristics were elucidated by next-generation sequencing, and the prognostic characteristics were analyzed.

Results: Epidermal growth factor receptor (EGFR) mutations were identified in 11/17 (65%) of patients. TP53 alterations were identified in 10/17 (59%). Other common mutations include ATM (18%), KRAS (18%), SDHA (18%), and TERT (18%). MPPAC patients harboring EGFR and TERT mutations were at a high risk of tumor recurrence, while TP53 might be associated with a low risk of recurrence.

Conclusions: TERT mutation was more frequently harbored in MPPAC patients than in the other histological type of lung cancer, and such patients were at a high risk of recurrence. So TERT mutation might be associated with adverse prognosis in MPPAC patients.

Background

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. Adenocarcinoma (AC) is the most common histological type of lung cancer. Lung AC with a micropapillary pattern (MPPAC) is determined as a histological subtype of AC according to the new multidisciplinary classification in 2011 [2]. It is characterized by a specific primary histological pattern, MPP, observed semiquantitatively in 5% increments on resection specimens. As a major component, tumor cells growing in papillary tufts form florets that lack fibrovascular cores in this variant of carcinoma [3]. These tumor cells may appear detached from and/or connected to alveolar walls and are usually small and cuboidal with variable nuclear atypia. Ring-like glandular structures may float within alveolar spaces. The vascular and stromal invasion is common, and also psammoma bodies are detected. The clinical manifestations of MPPAC patients are similar to those of other ACs [4] but have a high rate of lymphatic invasion [5–7], visceral pleural invasion [8–11], and lymph node metastases [4, 5, 9, 11–15]. Furthermore, most MPPAC patients are males and nonsmokers [9, 12, 16–19]. Comparing with other ACs, the molecular markers of MPPAC, including vimentin, napsin A, phosphorylated c-Met, cytoplasmic maspin, Notch-1, MUC1, and tumoral CD10, are highly expressed; conversely, markers such as MUC4 and surfactant apoprotein A have low expression in MPPAC [20–27]. MPPAC has a higher frequency of epidermal growth factor receptor (EGFR) mutations and v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations as compared to other histological subtypes [3, 22, 28–31], while the kirsten rat sarcoma 2 viral oncogene homolog (KRAS)

mutations or anaplastic lymphoma kinase (ALK) rearrangement are similar in all subtypes [22, 32–34]. Several studies have shown that MPP is associated with lung cancer prognosis, and even a minimal proportion of the tumor can lead to poor prognosis [7, 11, 12, 14, 35–37].

Methods

Sample collection

Formalin-fixed paraffin-embedded (FFPE) blocks from 17 MPPAC patients between 2012 and 2017 were collected retrospectively from Zhejiang Cancer Hospital in China. The pathological diagnosis was based on the standard criteria defined by the International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society (IASLC/ATS/ERS) [2]. The classification of stages was defined by the eighth edition of the TNM (tumor, node, metastasis) classification for lung cancer [38]. The clinical characteristics, such as gender, age, and clinical stage, are listed in Table 1. This study was approved by the Medical Ethics Committee of Zhejiang Cancer Hospital. All the specimens of the patients in this study were obtained from the Biological Sample Bank of Zhejiang Cancer Hospital, and the patients signed the written informed consent to preserve their specimens in the Biological Sample Bank of Zhejiang Cancer Hospital for research.

Table 1
Clinical characteristics of patients

Age at diagnosis (n = 17)	Years
Median	60
Range	47–70
Gender	No. of patients
Female	9
Male	8
Clinical stage	No. of patients
I	4
II	3
III	10

Next-generation sequencing (NGS)

The genomic alterations (GAs) in formalin-fixed, FFPE tissue samples obtained from 17 MPPAC patients were subjected to comprehensive profiling using NGS-based cancer gene panel, as described previously [39].

Results

Patient characteristics

8/17 MPPAC patients enrolled in this study were males. Of these, 7 were smokers, while none of the 9 female patients had a smoking history. The median age of the cohort was 60 (range, 47–70). 6/17 patients were diagnosed with stage I-II diseases, while 11 patients had stage III diseases. All patients were microsatellite stable (MSS), and no patient showed microsatellite instability-high (MSI-H).

Genomic alterations

In the current study, the most common mutations in these 17 MPPAC patients were as follows: EGFR (65%), tumor protein p53 (TP53) (59%), ataxia telangiectasia mutated (ATM) (18%), KRAS (18%), succinate dehydrogenase subunit A (SDHA) (18%), and telomerase reverse transcriptase (TERT) (18%) (Fig. 1a).

15/17 (89%) MPPAC patients harbored mutually exclusive mutations: 11 (65%) EGFR, 3 (18%) KRAS, and 1 (6%) BRAF, which was different from the rate of mutations in western countries. Achcar et al. [40] analyzed the molecular profile of 15 primary MPPAC patients with respect to KRAS, EGFR, and BRAF mutations and found that 11/15 (73%) MPPAC patients harbored mutually exclusive mutations: 5 (33%) KRAS, 3 (20%) EGFR, and 3 (20%) BRAF. This profile confirmed that the EGFR mutation rate is high in patients with MPPAC, but that the KRAS mutation rate is low in the Chinese population. Similar results were reported by other investigators. Chao et al. [41] and Zhang et al. [42] also found that micropapillary predominant subtypes are likely to occur with EGFR-mut tumors in Chinese lung ACs. Most studies from East Asia have reported a much lower rate of KRAS mutations than Western countries [43, 44]. In this study, the EGFR mutation rate is highest; 8/17 MPPAC (47%) patients presented an EGFR-sensitive mutation (EGFR 19del or L858R). The T790M and insertion mutations in exon 20 (E20ins) were the EGFR-tyrosine kinase inhibitor (TKI)-resistant mutations, among the 8 patients harboring an EGFR - sensitive mutation, 1 patient at the same time featured a EGFR-TKI-resistant mutation. 2/17 MPPAC (12%) patients featured a de novo EGFR-TKI-resistant mutation, and other 2/17 MPPAC (12%) patients featured less frequent mutations, such as G719X and L861Q. Another study showed a high EGFR-sensitive mutation rate in patients with MPPAC. A total of 211 patients with invasive AC were observed; 58 (27.5%) ACs featured MMP, 46 (79.4%) featured an EGFR-sensitive mutation (deletion in exon 19 or L858R). Interestingly, 5 (8.6%) MPPACs presented a de novo EGFR-TKI-resistant mutation, and 2 (12%) MPPACs featured less frequent mutations, such as G719X, L861Q, and S768I [45].

TP53 mutation is one of the most common genetic abnormalities found in all types of human tumors and has been reported in approximately half of the lung cancer [46]. In various malignancies, TP53 mutations have been associated with tumor progression, metastasis, resistance to chemotherapy and radiation, and reduced overall survival (OS) [47, 48]. Quinlan et al. [49] first reported TP53 oncoprotein expression as a poor prognostic factor in non-small cell lung carcinoma (NSCLC). The percentage of TP53 mutation varies by tumor type and ranges from 10–80% (<http://p53.free.fr>). In previous studies, TP53 alterations, mainly missense mutations, are found in 35–55% of NSCLC and are more prevalent in squamous cell carcinoma than AC [50, 51]. In this study, the mutation frequency of TP53 was similar to that observed previously. Herein, we detected TP53 mutations in tumor samples from 10/17 (59%) MPPAC patients.

Also, we identified that TERT frequently mutated in MPPAC patients. 3/17 (18%) MPPAC harbored TERT mutation. TERT activities are frequently upregulated in many human cancers and might contribute to human tumorigenesis [52]. The prevalence of TERT promoter mutations (pTERTm) was first identified in melanoma and subsequently detected in bladder cancer, glioblastoma, thyroid cancer, and other cancers [53–57]. pTERTm is a moderately prevalent genetic event in NSCLC. Also, the prevalence and association of pTERTms in NSCLC patients have been studied. Yuan et al. [58] identified a low frequency of pTERTm (5.8%) in NSCLCs, and found that patients who carry pTERTm are older than noncarriers, male patients were more likely to carry pTERTm and that pTERTm had a significant association with distant metastasis. Hence, it could be a poor prognostic factor for cancer patients. Ma et al. [59] demonstrated that 2.67% of NSCLC patients in their cohort had pTERTm, and those with TERT promoter mutation were significantly associated with older age. We also found that the mutation frequency of TERT was higher than that reported previously. However, due to the small sample size, we could not link TERT promoter mutations to the age and gender of patients.

The tumor mutation burden (TMB) is 0.7–24.7 (median 5.7) in these patients (Fig. 1b). According to the progression-free survival (PFS) results of Checkmate 026 [60] and 227 [61], TMB was considered as a potentially new and independent biomarker, the high TMB patients could choose nivolumab plus ipilimumab combination treatment, irrespective of PD-L1 expression level. TMB-positive advanced NSCLC patients who have no oncogenic drivers choose the immune checkpoint inhibitors as first-line treatment, while platinum-based chemotherapy may represent only the first-line option for patients with no PD-L1 expression and TMB-negative [62]. Nevertheless, the predictive value of TMB should be further investigated in future randomized trials. In the current study, the TMB was not significantly associated with disease-free survival (DFS). DFS was defined as the interval from the surgery to the point of any definite clinical or pathological evidence of local or distant disease recurrence or last evaluation.

Genomic alterations associated with the risk of recurrence

In this study, all patients were subjected to surgically and pathologically determined stages I, II, and III, which accounted for 4 (23%), 3 (18%), and 10 (59%) of the cohort. Thirteen patients received adjuvant chemotherapy, local radiotherapy, and EGFR TKIs after surgery. These patients presented different genomic alterations, and the DFS in patients ranged from 13 to > 72 months (alive at the time of testing) after diagnosis (Fig. 1c), suggesting that their genetic background might be related to the differences in their prognosis.

Some studies reported a higher than usual incidence of EGFR mutations in papillary and micropapillary AC of the lung [63, 64]. In this study, 11/17 (65%) MPPAC harbored EGFR mutations. Three cases with EGFR mutation were at stage I disease, 1 case was at stage II disease, and 7 cases were at stage III. The 2stage I patients received no other treatment after surgery, 1 stage II patient received chemotherapy, and 1 stage III patient received no other treatment after surgery, 1 received TKI treatment, and 5 received chemotherapy. The DFS of the patients received TKI treatment for > 46 months (alive at the time of the return visit), while the DFS in patients who received chemotherapy ranged from 13 to > 42 months. MPPAC patients harboring EGFR mutation might be good candidates for the treatment with EGFR TKIs. According to the recurrence status, 17 patients were divided into two groups: recurrence and non-

recurrence. One patient was lost to follow-up, and disease recurrence was found in 10 patients; 8/10 carried the EGFR mutation. Thus, MPPAC patients harboring the EGFR mutation might face a high risk of tumor recurrence. However, due to the small sample size, we did not observe a significant association between the risk of recurrence and EGFR mutation ($P = 0.299451$, Table 2). Kishi et al. [65] demonstrated that the MPPAC patients harboring L858R were at a high risk of recurrence in the pN0M0 lung AC. In previous studies, the correlation between EGFR mutations and the risk of recurrence of the MPPAC patients was controversial. Some investigators showed a positive correlation [63, 64, 66, 67], whereas one proposed that there was no correlation [12]. Sumiyoshi et al. [17] established a positive correlation between EGFR mutations and micropapillary component and speculated that MPPAC was biologically aggressive but could be controlled with EGFR-TKIs. The study recommended that to avoid the progression of the disease, the MPPAC patients harboring EGFR mutation should be actively treated with EGFR-TKIs. The current results showed that the EGFR mutation might be associated with the risk of recurrence in MPPAC patients. The risk of disease recurrence might be predicted by EGFR mutations, and the MPPAC patients harboring the EGFR mutation would be subjected to EGFR TKIs treatment.

Table 2
Correlation between EGFR and TERT mutations and the risk of tumor recurrence

Genes	MUT/recurrence N	MUT/non-recurrence N	WT/recurrence N	WT/non-recurrence N	Fisher's test P-value	Chi-sq. test P-value
TERT	3	0	7	6	0.25	0.408295
EGFR	8	3	2	3	0.299451	0.486234
Abbreviations: MUT, mutation; WT, wild type.						

In this retrospective study, we reviewed and analyzed the risk of recurrence in 17 MPPAC patients with documented tumor p53 mutational status (mutant-type [mtp53] vs. wild-type [wtp53]). Ten patients had mtp53, 1 was lost to follow-up, and 7 did not carry the TP53 mutation. 6/10 harbored the TP53 mutation showed a DFS of > 33 months, while 1/7 with wtp53 had a DFS > 33 months. Thus, TP53 could be speculated as a positive prognostic factor for enhanced DFS ($P = 0.06014$, Fisher's test), indicating that it is associated with a low risk of recurrence in MPPAC patients. However, due to the limited sample size, we did not observe a significant association between the risk of recurrence and TP53 mutation. In contrast to our data, a previous study showed that TP53 positive or overexpression is found to be significantly associated with short OS in NSCLC [68–70], and differences were observed between the various subtypes of NSCLC. Intriguingly, Nishio et al. [71] found an association between TP53 abnormalities and poor prognosis of patients with ACs but not with the squamous cell carcinomas. TP53 mutations are widespread in NSCLC, but to date, there are no approved agents that specifically target TP53 in NSCLC. The Wee-1 inhibitor AZD1775 is currently under investigation, but mainly with respect to small cell lung cancer [72].

In addition, 3/17 (18%) MPPAC patients harbored TERT mutation. BRAF and NRAS mutations activate the expression of TERT via MAPK pathway [54]. 68.6% of the lung ACs in the Chinese population carry EGFR or KRAS mutations [73], which were the known oncogenes that drive lung cancer by the MAPK/AKT pathway. In the present study, all 3 patients with TERT mutations had a concurrent EGFR mutation and also showed a disease recurrence. This phenomenon could be attributed to the TERT mutation in MPPAC patients, which elevated the risk of tumor recurrence in this group. However, due to the limited sample size, we did not observe a significant association between the risk of recurrence and TERT mutation ($P = 0.25$, Table 2). Another study showed that there was no significant difference in the OS or relapse-free survival (RFS) between TERT with and without mutation [54]. Therefore, additional studies and large sample sizes are needed to determine the correlation between TERT mutation and the risk of tumor recurrence. Previous findings of the associations of TERT expression and telomere length with the survival length of NSCLC patients also remained controversial. Several molecular epidemiology studies have associated TERT overexpression with poor prognosis in NSCLC patients [74–79]. Also, a significant association between high TERT levels and short periods of DFS and OS and high TERT levels in breast cancer were found to be significantly associated with short RFS [80]. A correlation between real-time quantitative measurement of TERT and the clinicopathological parameters of poor prognoses, such as histologic grade and muscle invasion, has been observed in bladder urothelial cell carcinomas [81], albeit with different conclusions [82–85]. The telomerase activity is one of the critical prognostic factors in NSCLC patients, as assessed by multivariate analysis. Additionally, TERT was not associated with the prognosis, as depicted in another study.

Discussion

Previous studies have demonstrated close correlations between the presence of MPP and lymph node metastasis, lymphatic invasion, venous invasion, differentiation grade, and TNM stage, suggesting that MPP display highly aggressive biological behavior. Thus, MPP in lung AC is considered as a distinct histopathological variant with biological and prognostic significance. The presence of an MPP could serve as an accurate indicator of the prognosis in lung AC patients.

In this study, we analyzed the genomic profiles of 17 MPPAC patients by targeted sequencing of pan-cancer genes in archived primary FFPE tissue samples. Thus, we obtained an overall profile of the genomic alterations for this distinctive subtype of invasive lung ACs and analyzed the prognostic characteristics of these patients. The mutational profiles of MPPAC patients were compared to that from a previous study on lung ACs. It was found that these distinctive micropapillary subtypes had similarities but also differed in genomic alterations as compared to those with lung ACs. *EGFR* was the most commonly mutated gene. TP53 alterations were more prevalent in MPPAC than lung ACs. The mutation rate of *KRAS* gene was similar in MPPAC and lung ACs, while the mutation frequency of TERT was higher than that reported previously.

Herein, we investigated the effect of EGFR, TP53, and TERT mutations on DFS in MPPAC patients. In the 17 MPPAC patients, disease recurrence was found in 10 patients; of these, 8 harbored the EGFR mutation

placing them at a high risk of tumor recurrence. Also, the MPPAC patients harboring TERT mutation were at similar risk, while TP53 mutation served as a positive prognostic factor for improved DFS. However, large sample size is required to confirm the above conclusion.

Conclusions

MPPAC is determined as a new histological subtype and a highly malignant potential type. TERT mutation was more frequently harbored in MPPAC patients than the other histological type of lung cancer, and those harboring TERT mutation were at a high risk of recurrence in patients. Thus, TERT mutation might be associated with the adverse prognostic in MPPAC patients. In the current study, EGFR, TP53, and TERT were the common mutations in MPPAC patients, and recognition of the common mutations would guide the treatment and predict the outcomes in MPPAC patients. According to targeted therapy, MPPAC patients harboring EGFR mutation were promising candidates for the treatment with EGFR TKIs. However, the high prevalence of TP53 and TERT mutations in MPPAC poses a challenge because of the absence of effective therapy for TP53 and TERT mutations.

Declarations

Disclosure

The authors report no conflicts of interest in this study.

Acknowledgments

This study was supported by the Zhejiang Province Medical Science and Technology Project (No. 2019ZH019).

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Figures

disease recurrence and black bars indicate non-recurrence. One patient was lost to follow-up and his DFS time was marked as “NA”. All patients were placed in the same order in the 3 panels.