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Comparative study of *EGFR* mutations detected in malignant pleural effusion, plasma and tumor tissue in patients with adenocarcinoma of the lung

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ABSTRACT

Objectives: The utility of malignant pleural effusion (MPE) as a source for determining *EGFR* mutations to guide EGFR TKI therapy in advanced adenocarcinoma of the lung remains unclear. This study compared MPE, plasma and tumor tissues as sources of biological samples for EFGR mutational analysis of lung adenocarcinoma patients.

Materials and methods: Total 295 MPE samples were retrospectively collected from lung adenocarcinoma patients. Matched tissue and plasma samples were available for 92 patients, and 248 patients had plasma samples. *EGFR* exon-19-deletion and exon 21-L858R mutation were detected with Denaturing high performance liquid chromatography (DHPLC). The concordance of *EGFR* mutation status in MPE, tissue, and plasma were evaluated, and the value of *EGFR* mutations in MPE with respect to efficacy of EGFR-TKI was investigated.

Results: The *EGFR* mutation rate in MPE samples was 39.3% (116/295). The concordance between MPEs and tissues was 87.1% (Kappa = 0.71); the sensitivity and specificity of *EGFR* mutation in MPEs according to tissues was 71.4% and 96.5%, respectively. And 219 patients received EGFR-TKI, and the objective response rate was similar for patients with *EGFR* mutation either in MPE, tissues or plasma (57.6% vs 56.0% vs 47.4%, p = 0.51). Similar results were found in progression free survival (8.9 months vs 9.0 months vs 7.7 months, p = 0.077 and overall survival (29.8 months vs 25.9 months vs 25.3 months, p = 0.33).

Conclusion: MPE is a reliable surrogate for tumor tissue for identifying*EGFR* mutations. MPE could offer reference of *EGFR* mutation to EGFR-TKIs treatment decision for advanced lung adenocarcinoma patients even when tissue and plasma were available.

1. Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) treatment has significantly improved the prognosis of patients with advanced lung adenocarcinoma with *EGFR* gene mutations. Lung cancer tissue obtained by surgery or biopsy is commonly used for categorizing tumors for clinical decisions [2–4], however, because in patients considered for EGFR-TKI therapy present in advanced stages, diagnostic materials are often limited to small biopsies, which may contain insufficient tumor cells for molecular analysis [5], approaches to detect molecular markers from samples other than surgical tissue must be explored to select more patients who are likely to respond to TKI treatment [6,7].

Malignant pleural effusion (MPE) is a common complication of advanced NSCLC, and adenocarcinoma is the most common cell type [8]. Sampling of effusion fluid is minimally invasive, often easy, and sometimes necessary in patients with symptoms of pain or dyspnea. For many cases, MPEs may be the only obtainable specimen allowing for the collection of sufficient tumor cells for molecular analysis. Plasma has been introduced into clinic practice as a surrogate for tumor tissues in assessing microscopic residual disease and response to therapy because of the non-invasive collection procedure. Some studies have analyzed the *EGFR* mutation rate in pleural effusions and the

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relationship between the mutation rate and patient response to gefitinib [9–13]. However, no previous study has investigated the *EGFR* mutation rate in MPEs of lung adenocarcinoma patients and compared it to the mutation rate in specimens of original lung adenocarcinoma tissues and plasma. In addition, the sensitivity and specificity of *EGFR* gene mutation detection in MPEs compared with plasma remains unknown. Furthermore, the predictive value of *EGFR* mutation detected in MPE has not been evaluated.

The purpose of this study was to evaluate the concordance of *EGFR* exon 19 deletion and exon 21 L858R mutation status between MPEs and matched tissues; to investigate the sensitivity and specificity of MPE compared with tissues as well as its prediction value compared with tissues and plasma from patients with lung adenocarcinoma; and finally, to determine if MPEs could be a surrogate clinical reference for EGFR-TKIs treatment decision.

2. Methods

2.1. Patients and samples

We retrospectively studied advanced lung adenocarcinoma patients who developed MPEs at the time of diagnosis or during the treatment (Fig. 1). The inclusion criteria were as follows: 1. Patients who were pathologically diagnosed adenocacinoma; 2. Patients developed plural effusion at diagnosis or during treatment which were cytological proved malignant with adenocacinoma cells; 3. The malignant plural effusion were available for EGFR mutation detection. Totally 295 patients with lung adenocarcinoma were recruited, who provided adequate MPE samples in the Thoracic Medical Department, Peking University Cancer Hospital, China, from September 2007 to May 2014. The last follow-up date was in July 2014. All patients were diagnosed with lung adenocarcinoma based on pathologic evaluation, and their MPE samples were cytologically positive for lung adenocarcinoma. There were 92 matching tissue samples and 248 matching peripheral blood samples for *EGFR* mutation analysis. And 219 out of 295 patients with clinical information received EGFR-TKI treatment were included for efficacy analysis. Among those 219 patients, MPE of 119 patients were collected for EGFR mutation detection before commence of EGFR-TKI treatment. Responses of the patients treated with EGFR-TKI was evaluated in accordance with Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST1.1) [14] guidelines. Progression free survival (PFS) is defined as the time from EGFR-TKI treatment to disease progression or death from any cause. Overall survival (OS) is defined as the time of diagnosis for lung adenocarcinoma until death from any cause. At the time of statistical analysis, 189 patients have experienced progression. The time points for scans is: first scan at 1 month after EGFR-TKI treatment, the following scans were arranged every two months until disease progression or worsened symptoms.

All patients had signed written informed consent at the time of sample collection and scientific analysis of clinical anonymized data. This study was approved by the local ethics committees in Peking University Cancer Hospital, China.

2.2. EGFR mutation detection

MPE samples (20 mL) and blood samples (10 mL) were collected from each patient. MPE samples were shelved for 10 min after collection. Two millilitres of the pleural effusion was collected and separated for DNA extraction. All the MPE samples were cytologically positive for lung adenocarcinoma according to a cytological smear. 1 mL of MPE supernatant were centrifuged at 2000 rpm for 5 min at room temperature to remove the residual debris. The rest supernatant were for EGFR detection. Plasma samples were centrifuged at 1000 *g* for 10 min at room temperature within one hour of collection. 200 uL supernatant was collected for DNA extraction and genomic sequencing.

Denaturing high performance liquid chromatography (DHPLC) was applied to detect EGFR mutations in tumor tissue, MPE supernatant and plasma according to the previously described methods [15]. This polymerase chain reaction (PCR)-based assay is capable of identifying two of the most common types of EGFR mutations: deletion (delE746-A750) in exon 19 (E19) and L858R in exon 21 (E21). We performed DHPLC by using the "Transgenomic Wave Nucleic Acid Fragment Analysis System" with a DNASep column (Transgenomic, Omaha, NE). Triethylammonium acetate at 0.05% acetonitrile in 0.1 M (TEAA; eluent A) and 25% acetonitrile in 0.1MTEAA (eluent B) were comprised in the mobile phases. The PCR products of 21 were denatured at 95 °C for 5 min and were cooled to 35 °C at a rate of 1 °C per minute to allow formation of heterozygote DNA. The product of exon19 was not denatured. The flow rate was set at 0.9 mL/min, and an ultraviolet detector works at 260 nm. We identified the heterozygous profiles by visual inspection of the chromatograms based on the appearance of additional, earliereluting peaks. Homozygous profiles showed only one peak. Four plasmids that contained the deletion mutation (delE746-A751) in exon19, L858R point mutation in exon21, and wild-type exon19 and 21 sequences was used. Serial dilutions (50%, 25%, 12.5%, 6.25%, 3.125%, and 1.6% of mutant alleles) were used for analysis.

3. Statistical analyses

The χ^2 test was used for categorical variables. The concordance rate of *EGFR* mutations and Cohen's kappa coefficients were calculated between MPTTs and MPEs. Kaplan-Meier was used for survival analysis.

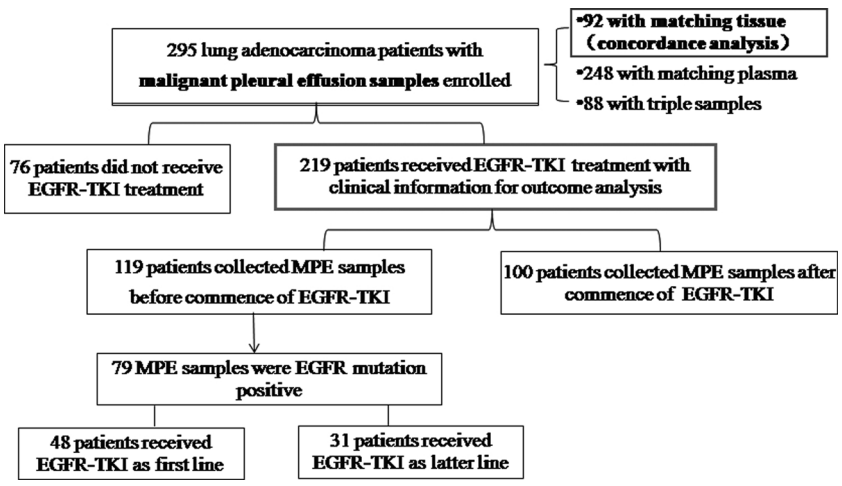

Fig. 1. Study Scheme.

Table 1
Clinicopathological characteristics of patients with MPEs (Total n = 295; EGFR mutant patient n = 116).

	Number of patients (%)	EGFR mutation (n/%)	P value
Sex			0.202
Male	156 (52.9)	56(35.9)	
Female	139 (47.1)	60(43.2)	
Age			0.039
> 65	106 (35.9)	50(47.2)	
< =65	189 (64.1)	66(34.9)	
Smoking history[a]			0.568
Active smoker	12 (4.1)	3(25.0)	
Ex-smokers	100(33.9)	39(39.0)	
Nonsmokers	183 (62.0)	74(40.4)	
Metastatic sites[b]			0.007
Brain	55(18.6)	31(56.4)	
Liver	29(9.8)	13(44.8)	
Both brain and liver	17(5.8)	9(52.9)	
Others	194(65.8)	63(39.3)	
M definition			0.137
M1a	126(42.7)	42(33.3)	
M1b	17(5.8)	9(52.9)	
M1c	152(51.5)	65(42.8)	

[a] Active smoker defined as someone who had smoked more than 100 cigarettes in their lifetime and who was currently smoking. Ex-smoker defined as someone who had smoked more than 100 cigarettes in their lifetime and who had quit smoking. Non-smoker defined as having either smoked 100 or fewer cigarettes in their lifetime or had never smoked cigarettes.

[b] Brain:metastatic sites include brain, liver excluded. Liver:metastatic sites include liver, brain excluded.Both brain and liver: metastatic sites include both brain and liver. Others:metastatic sites include bone, adrenal gland etc.

Two-sided p values < 0.05 were considered significant. The statistical analyses were carried out by SPSS 17.0.

4. Results

4.1. Patient characteristics

MPE samples were collected from 295 patients. 217 MPEs were identified at the initial diagnosis before any treatment, and 78 MPEs developed during the treatment. *EGFR* mutations were found in 39.3% (116/295) of MPE samples. Using DHPLC, we observed that the mutation rate of E19 (76/295, 25.8%) was higher than E21 (41/295, 13.9%), one patient was identified with concurrent E19 and E21mutations. As listed in Table 1, the mutation rate was more common in patients who were over 65 years old (47.2% vs. 34.9%, P = 0.039), and patients with brain metastesis compared with liver and other sites (56.4%, p = 0.007); it was similar between other different subgroups. Though due to the small number and being retrospective, the association of gender (female vs male: 43.2% vs 35.9%, p = 0.202) and smoking status (active smokers vs. ex-smokers vs. non-smokers: 25.0% vs 39.0% vs. 40.4%, p = 0.538) with EGFR mutation are not statistically significant.

4.2. The concordance of EGFR mutations in MPE, tissue and blood samples

295 patients with cytologically diagnosed MPE were available for analysis, of which 151 patients had *EGFR* mutation in either tissue or plasma or MPEs. Ninety-two patients provided adequate matched tumor tissue samples, and we obtained plasma samples from 248 of the 295 patients with MPE samples. *EGFR* mutation rates were 38.0% (35/92) in tumor tissue samples, 39.3% (116/295) in MPEs and 27.4% (68/248) in plasma. Compared to tumor tissues, the sensitivity and specificity of MPE samples for *EGFR* mutation detection were 71.4% (25/35) and 96.5% (55/57), and concordance between them was 87.1% (Kappa = 0.71). The positive predictive value (PPV) was 92.6% (25/27) and the negative predictive value (NPV) was 84.6% (55/65).

Table 2
Comparison of EGFR mutation detection in MPE and tissue.

	EGFR mutation in MPE		
EGFR mutation in tissue	+	–	Total
+	25	10	35
–	2	55	57
In total	27	65	92

Concordance 87.1% (Kappa = 0.71); sensitivity = 71.4%; specificity = 96.5%; PPV = 92.6%; NPV = 84.6%.

(Table 2).

4.3. Efficacy of EGFR TKI in patients with EGFR mutant MPE

Total 219 out of 295 patients received first generation TKI treatment and had complete follow-up data. Among those patients, 140 were treated with gefitinib, 63 received erlotinib and 16 received icotinib. For all patients with *EGFR* mutation in MPE who were treated with either first line or second line EGFR-TKI, the overall objective response rate (ORR) (56.0%, 56/100) and disease control rate (DCR) (94.0%, 94/100) were significantly higher compared with the wild-type group (19.3%, 23/119; P < 0.001; 63.0%, 75/119; P < 0.001). Also, the PFS was significantly shorter for EGFR wild type patients compared with EGFR mutant ones based on MPEs (3.3 months vs. 9.0 months; HR 1.67 (95%CI 1.208–2.330) ; P < 0.001) (Fig. 2a), and so was the OS (20.6 months vs. 25.9 months, HR 1.431 (95%CI 1.035–1.979) ; P = 0.032) (Fig. 2b).

We also analyzed the efficacy based on the EGFR mutation detected with tissues, MPEs and plasma. And results showed that for patients with EGFR mutations detected in tissue, the ORR, PFS and OS was 57.6%, 8.9 months, 29.8 months respectively. Similarly, for patients with EGFR mutations detected in MPEs, the ORR, PFS and OS was 56.0%, 9.0 months, 25.9 months respectively. The trend was also found in plasma, the RR, PFS and OS was 47.4%, 7.7 months, 25.3 months, respectively.(Fig. 3). And there was no difference in efficacy between groups of patients with EGFR mutation detected from various samples (Table 3).

We also found better efficacy in patients with E19 compared with E21 (ORR: 60.6% vs. 47.1%, P = 0.19; DCR: 97.0% vs. 88.2%; P = 0.08) treated with EGFR-TKI in all the MPE EGFR positive patients (Table S1), but the difference is not significant. A similar trendIn survival analysis, the OS of patients with E19 compared with E21 was somehow longer but without a significant p value (31.5 months vs. 21.8 months; HR1.485, 95%CI 0.841–2.624; P = 0.13) (Supplement data: Table S1 & Figure S1). However, there was no significant difference for PFS (9.1 months vs. 7.2 months; HR 1.645, 95%CI 0.97–2.791; P = 0.63).

We also analyzed the possible effect of subsequent therapy of those 106 patients with EGFR mutant MPEs after progression from EGFR-TKIs.

For patients treated with EGFR-TKI, EGFR sensitizing mutations of 56 patients were detected in MPEs only (tissue and plasma were both negative in 2 cases, tissue was not available in 54 cases, and plasma was not available in 16 cases). The ORR of these 56 patients treated with EGFR-TKI was 53.6%(30/56), and DCR was 94.6%(53/56). The median PFS and OS were 11.2 m and 24.6 m, respectively. (Fig. 4). In the two patients, whose EGFR mutation was only positive in MPEs instead of tissue and plasma, one of them was ECOG 3 at diagnosis, and died of pneumonia 2 weeks later; the other patient received EGFR-TKI as second line treatment, and the best response was PR, the PFS and OS was 13.5 months and 40.1 months respectively. Ten patients did not offer blood samples because they obtained EGFR mutation testing from MPEs first and started EGFR-TKI treatment.

We also collected the subsequent treatments of the patients after

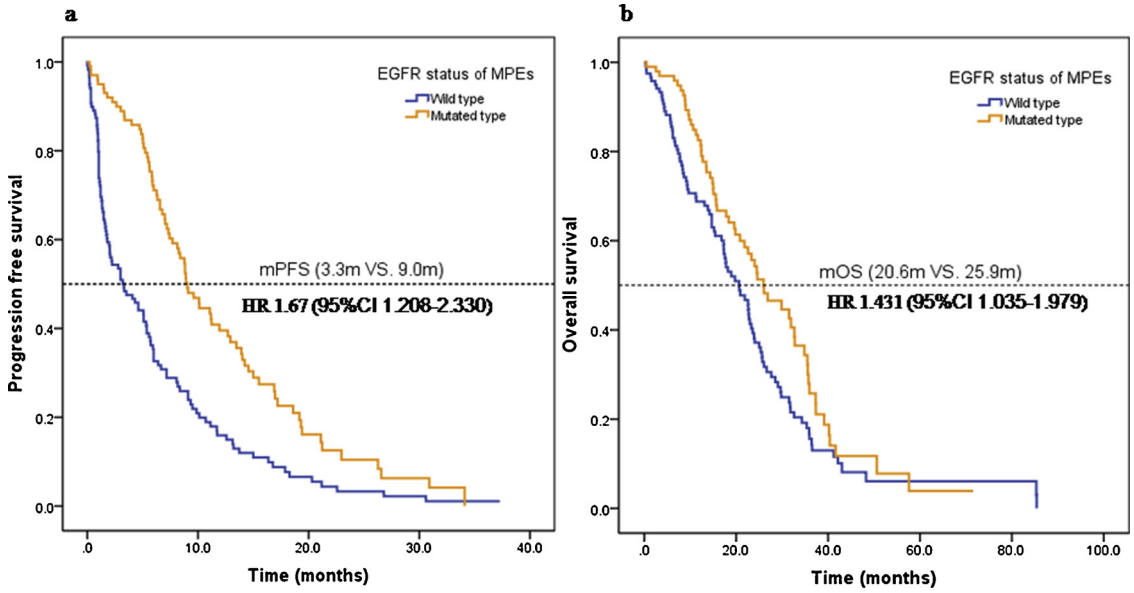

Fig. 2. PFS and OS according to EGFR status in MPEs with EGFR-TKI treatment: (a: left) Patients with EGFR mutation got longer PFS compared with EGFR wild-type patients (9.0 m vs. 3.3 m, $p < 0.001$). (b: right) In overall survival analysis, patients with EGFR mutation in MPEs had a significant longer OS compared with wild type patients (25.9 months vs. 20.6 months, P = 0.032).

progressing from EGFR-TKIs, and data from 151 of 219 pateints was available for analying (Table S2). Totally 9 patients revieved other TKIs including 1 st or 2nd generation EGFR-TKIs only, 41 patients got pemetrexed-based chemotherapy (single agent or combined with platinum), 9 patients received sequentioal TKIs and chemotherapy, 26 patients were treated with other chemotherapy (gemcitabine, taxanes, vinorelbine and irinotecan), and 66 patients had best supporteive care. The OS was not significantly different among those subgoups of patients as showed in Table S2 and Figure S2.

5. Discussion

To our knowledge, this is the first retrospective study to compare the EGFR mutation status from sampling tumor, plasma and MPE superantant and correlate results with efficacy of EGFR-TKI therapy. We identified a high concordance of EGFR mutation between tumor tissues and MPE superantant, which demonstrates the feasibility of using MPE superantant as a surrogate for tumor samples for EGFR mutation detection from lung adenocarcinoma patients. Of note, in this study, MPE superantant had a higher concordance with tissue and sensitivity than plasma, suggesting that it may be the better option for clinical testing when MPE superantant and plasma are both available, however, as DHPLC is probably not sensitive enough to detect plasma mutation and actually the standard techniques use either droplet-digital PCR-based assay or NGS (next generation sequencing), it is still too early to make thus conclusion which needs more data to confirm. Additionally, our data suggest that EGFR mutation in MPE superantant is a reliable predictive biomarker of EGFR-TKI efficacy that compares

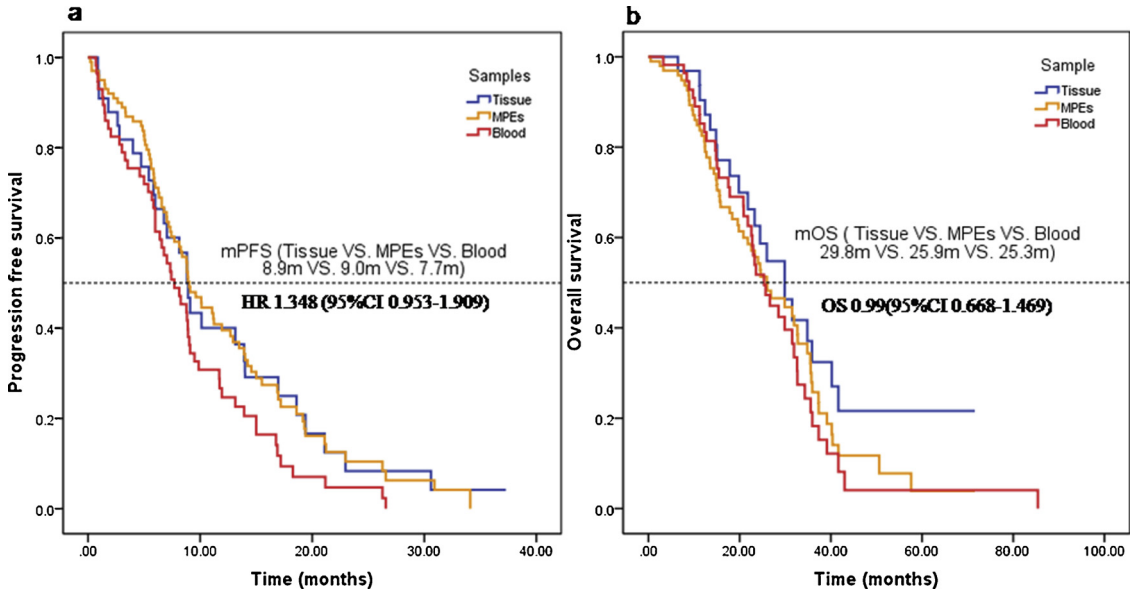

Fig. 3. Comparison of PFS and OS for EGFR mutant patients detected in different samples (tissue, MPE and plasma): For 219 patients received EGFR-TKI (a: left) The progression free survival (PFS) of patients with EGFR mutations was similar for patients with EGFR mutation either in MPE, tissues or plasma (8.9 months vs 9.0 months vs 7.7 months, p = 0.077). (b: right) The overall survival (OS) of patients with EGFR mutations was similar for patients with EGFR mutation either in MPE, tissues or plasma (24.0 months vs 25.9 months vs 22.9 months, p = 0.26).

Table 3
Efficacy and survival data of EGFR-TKI treatment based on different clinical samples.

Samples (number)	EGFR + number	RR%	P value	DCR%	P value	PFS m	P value	OS m	P value
Tissue (72)	27	57.6	0.51	87.9	0.13	8.9	0.077	29.8	0.33
MPE (219)	100	56.0		94.0		9.0		25.9	
Plasma (161)	53	47.4		84.2		7.7		25.3	

favorably with tissue and plasma.

A key finding in this study was that the concordance of EGFR mutation in MPE superantant and tissues was high (87.1%, Kappa = 0.71). This result is similar or even slightly better than previous reports [12,13]. This may be attributed to the fact that the MPE samples in our study were all cytologically positive with malignant cells. Compared to tumor tissue, the sensitivity and specificity were 71.4% (25/35) and 96.5% (55/57) for MPE supernatant; the PPV and NPV was 92.6% and 84.6% respectively. Liu et al reported a higher mutation rate in primary tumor samples compared with pleural effusion (61.98% vs. 58.85%). In our study, the results were, 38.0% in tissues and 39.3% in pleural effusion. However, in both studies, the difference was not significant. With a high concordance, specificity and moderate sensitivity, as well as the comparable EGFR mutation rate, MPE supernatant appears to be a reliable source of tissue for EGFR detection in clinical practice, when tumor tissue or MPE cell block are unavailable. When one considers that MPE samples represent tumor tissue, which can be abundant as opposed to the sometimes-scant numbers of circulating tumor cells or cell-free tumor DNA utilized from blood samples, it is not surprising that MPE supernatant samples that are positive for malignant cells will be a reliable source of tissue for EGFR genotyping. The specificity of MPEs was 96.5% for EGFR mutation detection compared to tissues, so there were 3.5% of patients (n = 2) may have false positive results. One of these patients chose EGFR-TKI as first line treatment according to the positivity of EGFR mutation, however this patient was ECOG 3 at diagnosis, and died of serious pneumonia 2 weeks later. The other patient received EGFR-TKI as third-line treatment, and the PFS was 11months, OS was 40 months suggesting she truly benefit from EGFR-TKI and cannot be explained as false positive. Besides, the EGFR mutation rate was as high as 39.3% in pleural effusion, for all the patients were Chinese, so validation studies are necessary in a Caucasian population with lower incidence of EGFR mutations (around 10%) [16–18].

This study represents the largest cohort of MPE samples studied to date. The EGFR mutation rate of 39.3% which is differenct from that of several previous studies. Gow et al and Soh et al [10,19] reported an EGFR mutation rate of 24.5% (13/53) in MPEs from lung adenocarcinoma. Kimura et al [11] identified a 13% (3/23) EGFR mutation rate by direct sequencing of MPEs from lung adenocarcinoma. On the other hand, Wang et al found that after enrichment by depletion of leukocytes, EGFR gene mutations were increased to 42.9% from 28.6%. Overall, the reported EGFR mutation rate of MPEs has ranged from 9.1% to 68.4%. The mutation rates may differ based on tumor histology, stage, time of sample collection, assay methodology, as well as patient ethnicity. EGFR mutations are more frequently observed in lung adenocarcinomas, and there is a high mutation rate in the Asian population. In terms of detection method, we utilized DHPLC, which is a reliable method for ctDNA based EGFR mutation testing with a detection limitation of 3% [15]. In a recent study, Yang et al. reported that EGFR mutations were detected in 72.5% of lung adenocarcinoma-associated MPE cell blocks (29/40). In our study, the EGFR mutation rate of patients in whom MPE supernatant samples were obtained before EGFR-TKI treatment was 66.4% (79/119), comparable to that of the MPE cell block. We do think MPE sampling that collects 20 mL of fluid as done in our study is more convenient than an MPE cell block and it also saves samples for other procedures. Also, the currentstudy was a retrospective analysis and the results need to be further confirmed.

The second important finding of the current study was that EGFR mutation in MPEs could predict efficacy of first generation EGFR-TKI treatment. As we know, EGFR mutations are a predictor of a better clinical outcome after TKI treatment in previous studies, all data suggested that EGFR mutation screening using MPEs from lung cancer patients may be useful for determining the need for TKI administration. For all the patients with EGFR mutation in MPEs in our study who were treated with either first line or second line EGFR-TKI, the ORR and DCR were 56.0% and 94.0% respectively, consistent with results seen from tissue-based assays and significantly higher than patients with wild-

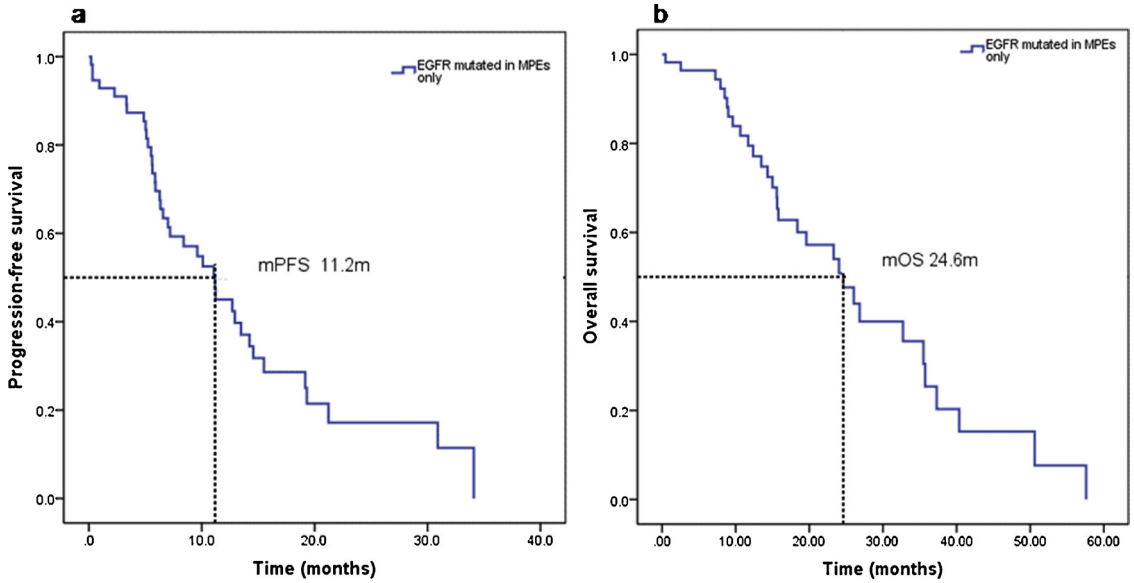

Fig. 4. PFS and OS of EGFR-TKI treatment in 56 patients with EGFR mutation only in MPE: In 56 patients out of which EGFR mutations were detected in MPEs only, a. the PFS was 13.5 months; b. the OS was 40.1 months.

type EGFR. Also, the PFS and OS were significantly longer for patients with EGFR mutant MPEs (9.0 months vs. 3.3 months; P < 0.001), and so was the OS (25.9 months vs. 20.6 months, P = 0.032).

We also compared the efficacy based on the EGFR mutation detected with tumor tissues, MPE supernatant and plasma. The results demonstrated that for patients with tissue-based EGFR mutations, the RR, PFS and OS were 57.6%, 8.9 months, 29.8 months respectively. Similarly, for patients with MPE supernatant-based EGFR mutations, the RR, PFS and OS were 56.0%, 9.0 months, 25.9 months respectively. The trend was also found in plasma, the RR, PFS and OS were 47.4%, 7.7 months, 25.3 months, respectively. And there was no difference in efficacy between groups of patients with EGFR mutation detected by various samples. Similar results were demonstrated in the survival analysis. This result is in accordance with our previous study which found high sensitivity and specificity of EGFR detaction in plasma and can be used as a biomarker to predict tumor response to TKIs [15].

Furthermore, we also found a trend of better efficacy in patients with E19 compared with E21 in both ORR (60.0% vs. 47.1%) and DCR (97.0% vs. 88.2%) as well as PFS (9.1 vs. 7.2 months) and OS (31.5 vs. 21.8 months), however the difference was not significant.

Meanwhile, In our study, the mutation was more common in women (43.2% vs. 35.9%, P = 0.202), but the difference didn't reach the statistical significance. The possible reason might be that all the consecutive patients included into this study are adenocarcinoma and most of whom are non-smokers (62.0%), which might eliminate the influence of gender and smoking status on EGFR mutation. For the result that older patients were more likely to obtain EGFR mutation, it was reported by previous study [20]. And this result could also attribute to the fact that this was a single center and retrospective study.

Finally, our study demonstrated that the predictive value of EGFR mutation in MPE supernatant might be superior to plasma. The predictive value of EGFR mutations has been extensively explored in plasma and various methodologies have been used to detect the plasma EGFR mutation status in NSCLC patients [15,21]. In previous studies, it has been reported that the ctDNA based EGFR mutation rate was between 23.7% and 55.8% in an unselected cohort of NSCLC patients [22–24]. In the current study, we assessed EGFR mutations in the plasma using DHPLC, and the rate of 27.4% was lower than what was found in MPE supernatant. So was the concordance, sensitivity and specificity compared with tissues. This suggests MPEs might be a better option for EGFR mutation detection than plasma, when both samples are available.

Potential explanations are that first, all MPE samples were confirmed cytologically to contain tumor cells and ctDNA concentration in plasma was not guaranteed. Secondly, the timing of MPE and plasma collection could be a factor. In 91 cases MPEs and plasma samples were collected at different time points. We are now prospectively collecting blood samples and MPEs at the same time point before the commencement of first line EGFR-TKI, with the aim of verifying the results of this study.

There are several limitations to our study. First, this is a retrospective study, and DHPLC was applied to detect all the samples including plasma, tissue and MPEs. It is probably not sensitive enough to detect plasma mutation compared with the standard techniques use either droplet-digital PCR-based assay or NGS. Also, DHPLC could only detect deletion (delE746-A750) in exon 19 and L858R in exon 21. The clinical relevance of some uncommon *EGFR* mutation variants remains uncertain and requires further assessment. Second, the time points of sample collection from tumor tissue, plasma and MPEs were not completely identical which might lead to the inconsistancy or the different EGFR mutation status between different types of samples. Third, the cellularity (mean or median number of cancer cells per mL effusion) was not calculated in this study, and we only have a pathological report demonstrating the positive cancer cells. This may lead to the false negative EGFR mutation due to the limited number of cancer cells in some of the MPEs samples. Last, as we showed in the results part, total 56 patients only had detectable EGFR mutation in MPEs, there might be some ethics issues since there was no strong evidence showed that MPEs based EGFR mutation could direct TKI treatment. However, based on the following aspects that: 1. there were some evidence from several retrospective studies that EGFR mutation rate in MPEs was concordant with that of tissues; 2. EGFR-TKI was widely applied as second line therapy in EGFR wild type lung cancer patients at the early time, and our study started from 2007; 3. All the MPEs samples were pathologically proved to be adenocacinoma cell positive, and most importantly, tissues were not available for detection.

6. Conclusion

MPE is a reliable surrogate for tumor tissue for identifying *EGFR* mutations. MPE could offer reference of *EGFR* mutation to EGFR-TKIs treatment decision for advanced lung adenocarcinoma patients even when tissue and plasma were available.

Declarations

- Ethics approval and consent to participate: We declare this study has Ethics approval and all the authors agree to participate.
- Consent for publication: All the authors agree to publish the study in Journal of Experimental & Clinical Cancer Research.
- Availability of data and material: Yes.
- Competing interests: None.
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- Authors' contributions: Shuhang Wang, Jun Zhao, and Jie Wang designed the study; Shuhang Wang, Hanxiao Chen and Hua Bai performed the experiment and analyzed the data, Shuhang Wang, Hanxiao Chen Jia Zhong, Haifeng Qin, Hua Bai and Jie Wang composed the manuscript.
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Availability of data and materials

The datasets used and/or analysed are available upon request as recommended by international statements from Editors of all big medical journals, without any restriction.

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Conflict of interest statement

All the authors declare No conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.05.018.

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