Respiratory Medicine Case Reports 29 (2020) 101007

Contents lists available at ScienceDirect

Respiratory Medicine Case Reports

journal homepage: http://www.elsevier.com/locate/rmcr

Use of a plasma test for verifying epidermal growth factor receptor gene (*EGFR*) mutations in fluid samples from non-small cell lung cancer patients☆

Wataru Ogura [a], Kouki Ohtsuka [b,*], Masachika Fujiwara [c], Ryota Tanaka [d], Kumiko Sekiguchi [a], Hiroaki Ohnishi [b], Takashi Watanabe [b]

[a] Department of Clinical Laboratory, Kyorin University Hospital, Tokyo, Japan
[b] Department of Laboratory Medicine, Kyorin University School of Medicine, Tokyo, Japan
[c] Department of Pathology, Kyorin University School of Medicine, Tokyo, Japan
[d] Department of Thoracic Surgery, Kyorin University School of Medicine, Tokyo, Japan

ARTICLE INFO

Keywords:
Non-small cell lung cancer
Epidermal growth factor receptor
Mutation
Pleural effusion
Cerebrospinal fluid
Plasma

ABSTRACT

Because epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are effective in the treatment of non-small cell lung cancer (NSCLC) patients with *EGFR* mutations, it is critical to obtain accurate *EGFR* mutation test results. For NSCLC patients, *EGFR* mutation testing is performed using the commercial Cobas *EGFR* Mutation test v2.0, which can be used to analyze both formalin-fixed, paraffin-embedded (FFPE) tissue (FFPE test, or FT) and plasma samples (plasma test, or PT). Since primary tumor tissues are often unavailable from relapsed patients, fluid samples are often used for *EGFR* mutation testing, but they are often tested using the FT. Here, we report three cases in which *EGFR* mutations were detected using the FT with FFPE primary tumor tissue samples, but were not detected using fluid samples (two pleural effusion and one cerebrospinal fluid sample). Because the FT may not be capable of detecting *EGFR* mutations in fluid samples, we used the PT, which is more sensitive, to verify the presence of *EGFR* mutations using the same fluid samples. As expected, the PT detected the same *EGFR* mutations in fluid samples as the FT did in FFPE primary tumor tissue samples.

1. Introduction

In this era of molecularly targeted therapies, diagnosing the mutations of patients with non-small cell lung cancer (NSCLC) is as important as establishing patients' clinical and histological status in developing an optimal treatment program [1]. For example, epidermal growth factor receptor (*EGFR*) mutation tests identify cases that are likely to be sensitive to EGFR-tyrosine kinase inhibitor (TKI) treatment. Approximately 10 and 35% of patients with NSCLC in the USA and East Asia, respectively, harbor tumor-associated *EGFR* mutations [2,3]. The *EGFR* mutations most frequently identified in NSCLC cases, including deletions in exon 19 (19del) and the L858R substitution in exon 21, have been reported to confer a high level of sensitivity to EGFR-TKIs including gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib. Thus, by testing for *EGFR* mutations, NSCLC cases in which EGFR-TKIs are effective can be identified [4–8].

In NSCLC cases, *EGFR* mutation tests are generally performed using formalin-fixed, paraffin-embedded (FFPE) primary tumor samples collected at first diagnosis; such samples usually contain a sufficiently high proportion of tumor cells [9,10]. However, because primary tumor tissues are not typically present in patients undergoing disease relapse, fluid samples may be used for *EGFR* mutation testing instead [11,12]. Since fluid samples contain fewer tumor cells, more sensitive detection methods are needed to ensure accurate diagnoses [9].

Currently, commercial *EGFR* mutation test kits, such as the Therascreen *EGFR* RGQ PCR kit (Qiagen Manchester Ltd, Manchester, UK) and the Cobas *EGFR* Mutation Test v2.0 (Cobas Test) (Roche Molecular Diagnostics, Pleasanton, CA, USA), are approved for *in vitro* diagnostic (IVD) testing in clinical settings in many countries, including Japan [10]. The Cobas Test was designed to test both FFPE tissue samples (FT)

☆ Presented at The 63rd Annual Meeting of Japanese Society of Laboratory Medicine in Kobe, Japan, on September 3, 2016.

* Corresponding author. Department of Laboratory Medicine, Kyorin University School of Medicine, 6-20-2, Shinkawa, Mitaka, Tokyo, 181-8611, Japan.
E-mail addresses: kytrans@ks.kyorin-u.ac.jp (W. Ogura), kouki7@ks.kyorin-u.ac.jp (K. Ohtsuka), masachi@ks.kyorin-u.ac.jp (M. Fujiwara), ryota@ks.kyorin-u.ac.jp (R. Tanaka), kssekiguchi@ks.kyorin-u.ac.jp (K. Sekiguchi), onishi@ks.kyorin-u.ac.jp (H. Ohnishi), twatanab@ks.kyorin-u.ac.jp (T. Watanabe).

https://doi.org/10.1016/j.rmcr.2020.101007
Received 2 September 2019; Accepted 24 January 2020
Available online 27 January 2020

and plasma samples (PT) [13].

For several NSCLC cases, we performed an initial Cobas FT on FFPE primary tumor tissue samples, but at the time of recurrence (e.g., after EGFR-TKI treatment), we used the FT on fluid samples (pleural effusion and cerebrospinal fluid) because tissue samples were unavailable, owing to the invasiveness of such tissue collection. However, in a few cases in which *EGFR* mutations had been detected in initial tissue samples, the FT failed to detect the mutations in fluid samples collected from the same patients at recurrence. Since the results of these tests differed, it was necessary to verify the accuracy of the results obtained from the fluid samples.

We predicted that more sensitive tests would be needed to detect mutations in fluid samples, given their lower numbers of tumor cells, and that the PT of the Cobas Test could be used for this purpose. Accordingly, the PT was used to test the fluid samples of patients yielding discordant *EGFR* mutation results [13].

2. Methods

This study was approved by the ethics committee of the Kyorin University School of Medicine. We obtained informed consent from all patients for the use of samples. All samples were pathologically and cytologically diagnosed as containing lung adenocarcinoma cells. After diagnosis, DNA was extracted from FFPE tumor, pleural effusion (200 μL), or cerebrospinal fluid (600 μL) samples, using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). The pleural effusion and cerebrospinal fluid samples were analyzed with both the FT and PT versions, and the FFPE tumor samples were analyzed using the FT of the Cobas Test (Roche Molecular Diagnostics) and a Cobas z480 instrument (Roche Molecular Diagnostics), according to the manufacturer's instructions.

3. Results

From 393 lung cancer cases in which *EGFR* mutations were examined using the FT at Kyorin University Hospital from March 2014 to June 2017, fluid samples were collected at the time of relapse (e.g., after EGFR-TKIs treatment); both the FT and PT were performed on fluid samples from 19 cases. Of these cases, 3 were identified in which the initial FTs on primary tumor tissue samples detected *EGFR* mutations, but the FTs on fluid samples (two pleural effusion and one cerebrospinal fluid) failed to detect any mutations. This could lead to the use of incorrect treatments, as NSCLC with wild-type *EGFR* are not treated with EGFR-TKIs [4–8].

In these cases, we attributed the discrepancy between the FT with FFPE primary tumor tissue and fluid samples to the lower numbers of tumor cells in the fluid samples. Therefore, the PT, which is more sensitive than the FT, was used to verify the initial *EGFR* mutations [13].

The FT on primary tissue samples showed that one and two of the patients harbored 19del and L858R mutations, respectively. While the FT failed to detect *EGFR* mutations in the corresponding fluid samples, the PT on the fluid samples yielded results that were consistent with the FT on the primary tumor tissue samples (Table 1).

In 16 other cases, FTs on primary tumor tissue and fluid samples yielded consistent results: In 5 cases, we found 3 cases of 19del and 2 cases of L858R. In the 11 remaining cases, the FT was not performed on FFPE primary tissue samples, but the results of the FT and the PT on fluid samples yielded 9 cases with wild-type *EGFR* and 2 with 19del (Table 2).

Table 1
EGFR mutation tests.

			Fluid samples		FFPE tissue samples (primary tumor)
case	Sample type	Conc (ng/μL)	FFPE test (FT)	Plasma test (PT)	FFPE test(PT)
1	PE	295	WT	19del	19del
16	PE	227	WT	L858R	L858R
17	CF	10	WT	L858R	L858R

CF, cerebrospinal fluid; Conc, concentration, FFPE, formalin fixed and paraffin-embedded; PE, pleural effusion; WT, wild-type.

4. Discussion

To the best of our knowledge, this study is the first to employ the PT to clarify discordant FT results. These results indicate that PTs and FTs performed on the same fluid samples can disagree, but the PT on fluid samples yielded results consistent with results of the FT on FFPE primary tumor samples.

It is likely that the low numbers of tumor cells in the fluid samples made *EGFR* mutations undetectable by the FT and that the PT was successful with fluid samples because it is more sensitive than the FT. Before the clinical introduction of the PT, it would have been impossible to clarify such discordant FT results [13].

Therapies for lung cancer that target specific molecules are currently being developed, and treatments vary significantly depending on the whether the target molecule is involved. EGFR-TKIs, which are highly effective against *EGFR*-mutated lung cancer, should only be used if there has been a positive *EGFR* mutation test [4–8]. However, if *EGFR* screening is inaccurate, as in the cases investigated here, it is possible that patients will not be treated correctly, considering that wild-type *EGFR* patients are not generally helped by EGFR-TKIs [4–8]. Therefore, as demonstrated by our use of the PT, it is essential not only to develop, but to use more accurate diagnostic tests.

The PT has also been used to detect mutations in cell-free DNA (cfDNA) in plasma samples, which is significantly less invasive than collecting other fluid samples. However, plasma testing only detects *EGFR* mutations in 73% of the NSCLC patients for which *EGFR* mutations were detected by FTs of FFPE primary tumor tissue samples [14]. Fluid samples were used in the present study because it clearly is easier to detect mutations in fluid samples than in plasma samples, given that other fluid samples are known to contain tumor cells.

Because this study was limited by a small sample size, it is impossible to draw definite conclusions. However, in the near future, by the same types of studies, it likely will be confirmed that the PT is essential for detecting *EGFR* mutations in fluid samples in similar cases.

In conclusion, the PT, which is more sensitive than the FT for detecting *EGFR* mutations in fluid samples, might be useful for clarifying discrepancies between the results of the FT between FFPE primary tumor tissue and fluid samples.

Table 2
Results of *EGFR* mutation tests.

			Fluid samples		FFPE tissue samples (primary tumor)
case	Sample type	Conc (ng/μL)	FFPE test (FT)	Plasma test (PT)	FFPE test(PT)
2	PE	501	WT	WT	NA
3	PE	171	WT	WT	NA
4	PE	213	WT	WT	NA
7	PE	130	WT	WT	NA
8	PE	64	WT	WT	NA
9	PE	143	WT	WT	NA
10	PE	68	WT	WT	NA
13	PE	358	WT	WT	NA
14	PE	254	WT	WT	NA
6	PE	101	19del	19del	NA
11	PE	610	19del	19del	NA
5	PE	302	19del	19del	19del
12	PE	960	19del	19del	19del
15	PE	574	L858R	L858R	L858R
18	CF	8.6	L858R	L858R	L858R
19	CF	2	19del	19del	19del

CF, cerebrospinal fluid; Conc, concentration, FFPE, formalin fixed and paraffin-embedded; PE, pleural effusion; WT, wild-type; NA, not available.

Funding

This work was supported by JSPS KAKENHI Grant Numbers JP17K10799, JP16K10690.

Declaration of competing interest

None.

References

[1] T. Mitsudomi, Y. Yatabe, Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer, Canc. Sci. 98 (2007) 1817–1824, https://doi.org/10.1111/j.1349-7006.2007.00607.x.

[2] T.J. Lynch, D.W. Bell, R. Sordella, S. Gurubhagavatula, R.A. Okimoto, B. W. Brannigan, et al., Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib, N. Engl. J. Med. 350 (2004) 2129–2139, https://doi.org/10.1056/NEJMoa040938.

[3] J.G. Paez, P.A. Janne, J.C. Lee, S. Tracy, H. Greulich, S. Gabriel, et al., EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy, Science 304 (2004) 1497–1500, https://doi.org/10.1126/science.1099314.

[4] M. Maemondo, A. Inoue, K. Kobayashi, S. Sugawara, S. Oizumi, H. Isobe, et al., Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR, N. Engl. J. Med. 362 (2010) 2380–2388, https://doi.org/10.1056/NEJMoa0909530.

[5] R. Rosell, E. Carcereny, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, et al., Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial, Lancet Oncol. 13 (2012) 239–246, https://doi.org/10.1016/S1470-2045(11)70393-X.

[6] L.V. Sequist, J.C. Yang, N. Yamamoto, K. O'Byrne, V. Hirsh, T. Mok, et al., Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations, J. Clin. Oncol. 31 (2013) 3327–3334, https://doi.org/10.1200/JCO.2012.44.2806.

[7] T.S. Mok, Y. Cheng, X. Zhou, K.H. Lee, K. Nakagawa, S. Niho, et al., Improvement in overall survival in a randomized study that compared dacomitinib with gefitinibin patients with advanced non-small-cell lung cancer and EGFR-activating mutations, J. Clin. Oncol. 36 (2018) 2244–2250, https://doi.org/10.1200/JCO.2018.78.7994.

[8] J.C. Soria, Y. Ohe, J. Vansteenkiste, T. Reungwetwattana, B. Chewaskulyong, K. H. Lee, et al., Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer, N. Engl. J. Med. 378 (2018) 113–125, https://doi.org/10.1056/NEJMoa1713137.

[9] K. Goto, M. Satouchi, G. Ishii, K. Nishio, K. Hagiwara, T. Mitsudomi, et al., An evaluation study of EGFR mutation tests utilized for non-small-cell lung cancer in the diagnostic setting, Ann. Oncol. 23 (2012) 2914–2919, https://doi.org/10.1093/annonc/mds121.

[10] D.S. Tan, S.S. Yom, M.S. Tsao, H.I. Pass, K. Kelly, N. Peled, et al., The International Association for the Study of Lung Cancer consensus statement on optimizing management of EGFR mutation positive non-small cell lung cancer: status in 2016, J. Thorac. Oncol. 11 (2016) 946–963, https://doi.org/10.1016/j.jtho.2016.05.008.

[11] H.J. Yoon, H.Y. Lee, K.S. Lee, Y.L. Choi, M.J. Ahn, K. Park, et al., Repeat biopsy for mutational analysis of non-small cell lung cancers resistant to previous chemotherapy adequacy and complications, Radiology 265 (2012) 939–948, https://doi.org/10.1148/radiol.12112613.

[12] K. Nosaki, M. Satouchi, T. Kurata, T. Yoshida, I. Okamoto, N. Katakami, et al., Re-biopsy status among non-small cell lung cancer patients in Japan: a retrospective study, Lung Canc. 101 (2016) 1–8, https://doi.org/10.1016/j.lungcan.2016.07.007.

[13] H. Kimura, T. Ohira, O. Uchida, J. Matsubayashi, S. Shimizu, T. Nagao, et al., Analytical performance of the cobas EGFR mutation assay for Japanese non-small-cell lung cancer, Lung Canc. 83 (2014) 329–333, https://doi.org/10.1016/j.lungcan.2013.12.012.

[14] C. Karlovich, J.W. Goldman, J.M. Sun, E. Mann, L.V. Sequist, K. Konopa, et al., Assessment of EGFR mutation status in matched plasma and tumor tissue of NSCLC patients from a phase I study of rociletinib (CO-1686), Clin. Canc. Res. 22 (2016) 2386–2395, https://doi.org/10.1158/1078-0432.CCR-15-1260.