Respiratory Medicine Case Reports 22 (2017) 183–186

Contents lists available at ScienceDirect

Respiratory Medicine Case Reports

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

Case report

A case report of *EGFR* mutant lung adenocarcinoma that acquired resistance to *EGFR*-tyrosine kinase inhibitors with *T790M* mutation and epithelial-to-mesenchymal transition

Nana Zhang [a, b], Depu Wang [a], Xiaofeng Li [a], Zhe Yang [a], Guanjun Zhang [a], Yili Wang [b, *], Chunbao Wang [a, **]

[a] Department of Pathology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, China
[b] Institute of Cancer Research, School of Basic Medical Science of Xi'an Jiaotong University, Xi'an, 710061, China

ARTICLE INFO

Article history:
Received 29 May 2017
Received in revised form
14 August 2017
Accepted 16 August 2017

Keywords:
Epidermal growth factor receptor tyrosine kinase inhibitor
EGFR-TKI resistance
Secondary mutation
Epithelial-to-mesenchymal transition

ABSTRACT

Although a secondary mutation and the epithelial-to-mesenchymal transition (EMT) are encountered very often in patients received the *EGFR*-TKI targeted treatment. The entire detrimental morphological change of the cancer entity was rare reported. Herein we report a case that acquired resistance to *EGFR*-TKI with *T790M* mutation and complete EMT morphological change of the tumor tissue. The primary lung tumor from a 52-year-old woman was diagnosed with moderate differentiated adenocarcinoma, with intensively positiveTTF-1 and E-cadherin in differentiated glandular structure but not the budding cancer cell cluster which with an intensive Vimentin staining. Molecular analysis revealed an *EGFR* exon 19 deletion and with an excellent response to Gefitinib treatment. Microscopic examination of recurred tumor specimens revealed a diffuse poorer differentiated proliferation of atypical cells. Immunostaining showed intensive Vimentin but almost completely negative for E-cadherin and TTF-1. Genetic analyses revealed *T790M* mutation. It is worth noting that rare clinical studies have been reported that acquired *EGFR*-TKI resistant lung adenocarcinoma underwent *T790M* mutation and almost complete EMT together. More significantly, the similarity of poorly differentiated cancer cell cluster in the primary lesions to recurred tumor lesions, which may pre-harbor drug resistance mutation should not be neglected underneath the predominant morphologic patterns.

1. Introduction

Non-small-cell lung cancer (NSCLC) is the predominant major cause of malignancy related death. Although tyrosine kinase inhibitors (TKIs) targeting the epidermal growth factor receptor (EGFR) have shown effectiveness as therapy for advanced NSCLC with mutated *EGFR* gene, acquired resistance has emerged as a major limitation of *EGFR*-targeted therapies with TKIs [1–3]. The most important mechanisms of resistance is a secondary mutation in the *EGFR* gene, the *T790M* mutation in exon 20, responsible for about 50% of cases, and along with the epithelial-mesenchymal-transition (EMT) accompanied with the decreased efficacy of therapy [4,5]. Currently, the entity of cancer heterogeneity provides new clues to the TKI resistance [6]. In the routine practice, it is often to find minute poor differentiated cancer clusters that exhibit the EMT phenotype of which is ignored but the majority are differentiated lesions, which is reminded that the minority of the cancer cells with the preexistent instead of the acquired mutation may obtain the growth advantage under the pressure of targeted therapy [7,8]. Herein we report a case of moderate differentiated lung adenocarcinoma with EMT phenotypes in the infiltrating front that initially was *EGFR*-TKI sensitizing mutations and gained both resistant *T790M* mutation and largely EMT in the recurred lesion after gefitinib treatment. It is noteworthy to emphasize that although the preexistent *T790M* mutation was not detected in the primary tumor samples by the conventional technique before TKI treatment, the cluster of cancer cells with EMT phenotypes in the original lesion which are similar to that in recurred lesions should not be ignored when predicting the resistance acquisition in TKI

* Corresponding author. Institute for Cancer Research, School of Basic Medical Science of Xi'an Jiaotong University, Xi'an, 710061, China.
** Corresponding author. Department of pathology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, China.
E-mail addresses: wangyili@mail.xjtu.edu.cn (Y. Wang), binglizilliao2012@163.com (C. Wang).

http://dx.doi.org/10.1016/j.rmcr.2017.08.015

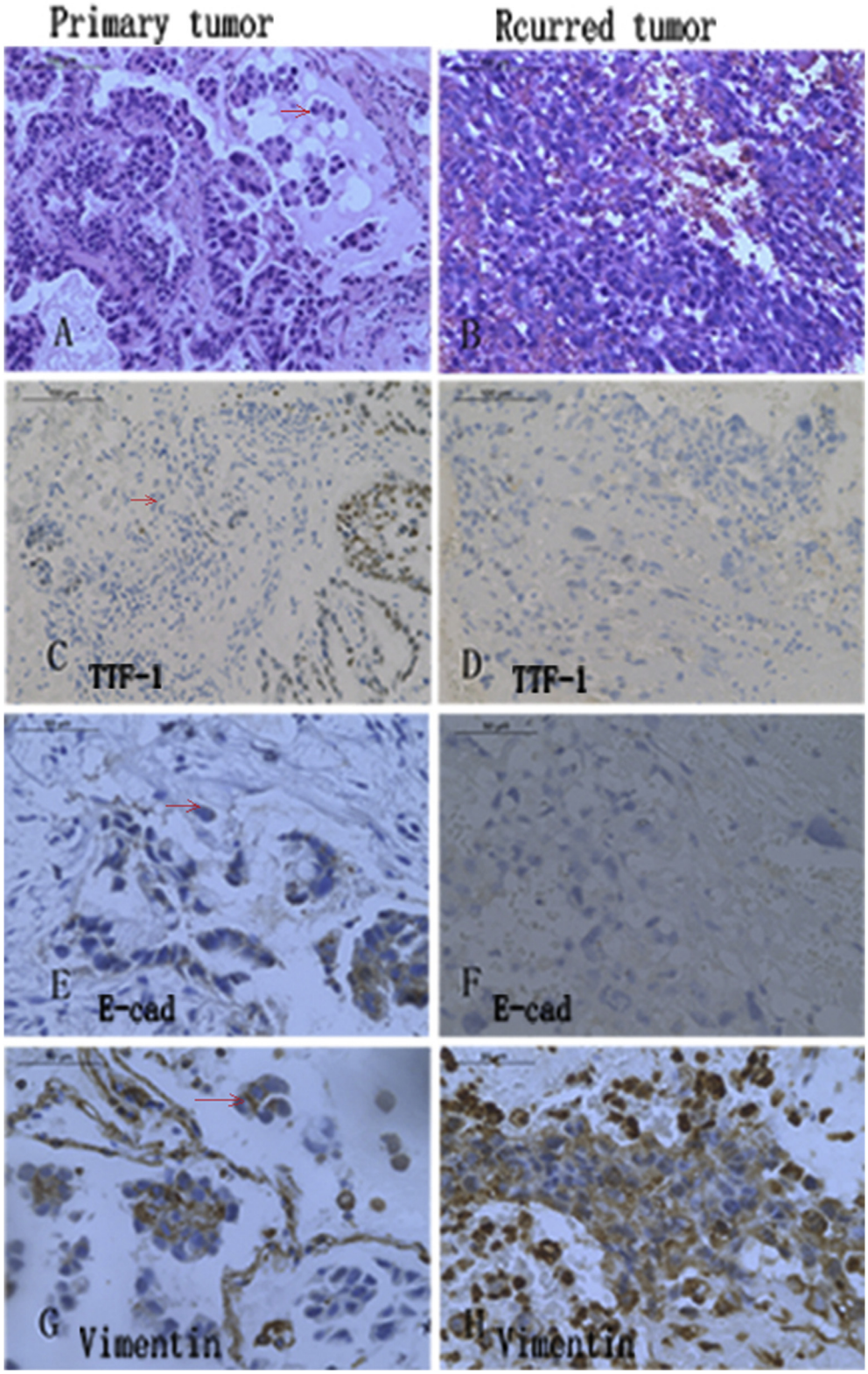

Fig. 1. The H&E staining tumor tissue and immunohistochemistry staining tumor tissues for TTF-1, E-cadherin and Vimentin phenotypes. The primary tumor tissues(A,C,E,G). The primary tumor showed moderate differentiated adenocarcinoma(A). The TTF-1 and E-cadherin were positive in the differentiated cancerous tissue but attenuated in the tumor cell clusters(which is indicated by the red arrow in the figure) in the invasive margin of the cancer(C,E). Vemintin were positive in the tumor cell clusters(indicated by the arrow) in the invasive margin of the cancer (G). The expectoration of recurred tumor tissue block(B,D,F,H). The recurred tumor tissue showed, TTF-1,E-cadherin and Vimentin stained sections. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatment [9].

2. Case report

A 52-year-old woman was diagnosed with moderate differentiated adenocarcinoma (T1NxM1a with visceral pleural nodules) harboring an *EGFR exon 19* deletion by computed tomography (CT)-guided lung tumor biopsy and subsequently exploratory thoracotomy. Microscopically, the primary tumor tissues exhibited moderate differentiated glandular and papillary patterns, the micropapillary and tumor cell clusters were scattered in the peripheral invasive margin (Fig. 1 A). TTF-1, E-Cadherin and Vimentin phenotypes were identified by immunohistochemistry. TTF-1 was positive in the differentiated glandular cancerous tissue but weak/negative in the tumor cell clusters in the invasive margin (Fig. 1 C, Table 1). E-cadherin were positive in the differentiated cancerous tissue but attenuated in the tumor cell clusters in the invasive margin too (Fig. 1 E, Table 1), while Vimentin was negative in the differentiated cancerous tissue but positive in the tumor cell clusters in the infiltrating margin of the cancer [Fig. 1G, Table 1]. Gefitinib was administered as first-line therapy, a well response was achieved. However, the patient underwent a regression but progressed after two years disease stabilization, Gefitinib therapy was stopped and followed by Pemetrexed plus platinum therapy and this approach was for one year. As for the recurred tumor specimens, tumor tissue in a clot-like expectoration of the patient were collected two months before the patient died. Surprisingly, the recurred tumor tissue revealed diffuse proliferation of atypical giant cells, almost no adenocarcinomatous components such as those glandular and papillary structures seen in the original specimens by H&E stained section. The cancer cells showed significant atypia, hyperchromatic staining and predominant nucleoli, more giant tumor cells and mitotic figures (Fig. 1B). And negative TTF-1, strong Vimentine but attenuated E-cadherin staining were demonstrated (Fig. 1H,F). These results suggested that the recurred lesions had undergone an EMT phenotypic transformation, the morphological patterns and phenotypic change were similar to the tumor cell clusters in the invasive margin of the primary lesions. As expectedly, the secondary *T790M* mutation in exon 20 was detected in the recurred lesions (Table 1).

3. Discussion &conclusion

The most prevalent mechanisms of acquired *EGFR*-TKI resistance are the *EGFR T790M* mutation, *PIK3CA* mutation, *MET* amplification and EMT [10,11]. In the present case, the molecular test for the primary tumor demonstrated the existence of TKI sensitive mutation and it indeed obtained good response to the Gefitinib administration. The presence of *T790M* mutation in the recurred lesions may explain the *EGFR*-TKI resistance reasonably because of the lacking of *T790M* mutation in the original lesions. Most obvious features of the recurred lesion were poorer differentiation with almost no identifiable adenocarcinomatous components and the E-cadherin expression was almost subsided and Vimentin expression was more intensity. All these seem to be consistent with the current consensus as mentioned above. Nevertheless, the relationship between the EMT status and *T790M* are unknown, some study showed that EMT plays roles in resistance to *EGFR*-TKI but independent of *T790M* mutation [12]. Generally, secondary *T790M* mutation and EMT phenomenon are considered separately in acquisition of *EGFR*-TKI resistance [13,14]. In the present case, there are two points being attracted. The first, whether the recurred lesions with EMT are resultant from selective amplification of the pre-existent cancer cell cluster with EMT under treatment. Apparently, the poor differentiated cancer cell clusters with EMT phenotype were detected in the original lesions, and similar morphology and phenotype exhibited in most of the recurred lesion too. In terms of the acquired TKI resistant *T790M* mutation, it may be harbored in the original lesion but at levels below the threshold of detection, and then may expand selectively under Gefitinib treatment, leading to the failure of Gefitinib therapy. And this resistance mechanism that the selected amplification of minority cancer cells with preexistence gene mutation under the TKI therapeutic pressure have being evidenced in some in vitro and in vivo experiments of NSCLC [7,8,15–18], which indicate that heterogeneous tumors inherently possess the potential to give rise to *EGFR*-TKI resistant cells with different resistance mechanisms depending on the treatment, and which has also been concerned and recognized for a long time [19,20]. It happens that there are similar comparable reports about the tumor lesion heterogeneity, in which the main points are that a single biopsy from a tumor might not be sufficient to give a full picture of its genetic landscape [6]. Therefore, considering the findings in the present case, it is not totally convinced that the morphological transformation of the tumor in original and recurred lesions is ascribed to the new acquired mutation, the fact that selective pressure of drug endow growth advantage to the cell population with pre-existing resistant mutation also is an account of the mechanism. It is extremely regrettable in this case, we did not obtain the solid evidence of pre-existing *T790M* mutation from the tumor cell cluster with EMT phenotype in the original lesion because of the shortage of the cell resource.

Taken together, it is worthy noting that when considering the acquired TKI resistance with new resistant mutation and EMT process, we need to pay attention to tumor heterogeneity within the tumor, and multiple biopsies (or micro-dissecting sampling) might solve the problem and give a full picture of its genetic landscape in order for the accurate targeting therapy.

Table 1
Analysis of genetic mutation and EMT markers in primary and secondary specimens.

Genetic mutation and EMTmarkers	Primary lesions		recurred lesions
	Moderate differentiated parts	The budding cancer cell cluster parts	
Exon-19/19-del	(+)	NA	(+)
Exon-21/L858R	(−)	NA	(−)
Exon-20/T790M	(−)	NA	(+)
Exon-20/20-ins	(−)	NA	(−)
Exon-18/G719X	(−)	NA	(−)
Exon-20/S768I	(−)	NA	(−)
Exon-21/L861Q	(−)	NA	(−)
E-Cadherin	(+++)	(−)	(+)
Vemintin	(+)	(+++)	(+++)
TTF-1	(++)	(−)	(−)

NA: not available.

Conflict of interests

The authors report no conflict of interests.

References

[1] K. Zhang, Q. Yuan, Current mechanism of acquired resistance to epidermal growth factor receptor-tyrosine kinase inhibitors and updated therapy strategies in human nonsmall cell lung cancer, J. Cancer Res. Ther. 12 (2016) 131–137, http://dx.doi.org/10.4103/0973-1482.200613.
[2] Z. Piotrowska, L.V. Sequist, Epidermal growth factor receptor-mutant lung cancer: new drugs, new resistance mechanisms, and future treatment options, Cancer J. 21 (5) (2015) 371–377, http://dx.doi.org/10.1097/PPO.0000000000000147.
[3] K. Suda, H. Mizuuchi, Y. Maehara, T. Mitsudomi, Acquired resistance mechanisms to tyrosine kinase inhibitors in lung cancer with activating epidermal

growth factor receptor mutation–diversity, ductility, and destiny, Cancer Metastasis Rev. 31 (34) (2012 Dec), http://dx.doi.org/10.1007/s10555-012-9391-7, 807e814.
[4] C. Ma, S. Wei, Y. Song, T790M and acquired resistance of EGFR TKI: a literature review of clinical reports, J. Thorac. Dis. 3 (1) (2011) 10–18, http://dx.doi.org/10.3978/j.issn.2072 -1439. 2010.12.02.
[5] J. Zhou, J. Wang, Y. Zeng, X. Zhang, Q. Hu, B. Xie, W.M. Zhang, Implication of epithelial-mesenchymal transition in IGF1R-induced resistance to EGFR-TKIs in advanced non-small cell lung cancer, Oncotarget 6 (42) (2015 Dec), http://dx.doi.org/10.18632/oncotarget.6293, 44332e44345.
[6] M. Gerlinger, A.J. Rowan, S. Horswell, M. Gore, Z. Szallasi, J. Downward, P.A. Futreal, C. Swanton, Intratumor heterogeneity and branched evolution revealed by multiregion sequencing, N. Engl. J. Med. 366 (10) (2012) 883–892, http://dx.doi.org/10.1056/NE JMoa1113205.
[7] Y. Lee, G.K. Lee, Y.S. Lee, W. Zhang, J.A. Hwang, B.H. Nam, S.H. Kim, J.H. Kim, T. Yun, J.Y. Han, J.S. Lee, Clinical outcome according to the level of preexisting epidermal growth factor receptor T790M mutation in patients with lung cancer harboring sensitive epidermal growth factor receptor mutations, Cancer 120 (14) (2014) 2090–2098, http://dx.doi.org/10.1002/cncr.28711.
[8] K.Y. Su, H.Y. Chen, K.C. Li, M.L. Kuo, J.C. Yang, W.K. Chan, B.C. Ho, J.Y. Shih, S.L. Yu, P.C. Yang, Pretreatment epidermal growth factor receptor (EGFR) T790M mutation predicts shorter EGFR tyrosine kinase inhibitor response duration in patients with non-small-cell lung cancer, J. Clin. Oncol. 30 (4) (2012) 433–440, http://dx.doi.org/10.1200/J CO.2011.38.3224.
[9] D. Ding, Y. Yu, Z. Li, X. Niu, S. Lu, The predictive role of pretreatment epidermal growth factor receptor T790M mutation on the progression-free survival of tyrosine-kinase inhibitor-treated non-small cell lung cancer patients: a meta-analysis, Oncol Targets Ther. 7 (2014) 387–393, http://dx.doi.org/10.2147/OTT.S58870.
[10] P. Ulivi, Non-invasive methods to monitor mechanisms of resistance to tyrosine kinase inhibitors in non-small-cell lung cancer: where do we stand? Int. J. Mol. Sci. 17 (7) (2016 Jul) e1186, http://dx.doi.org/10.3390/ijms17071186.
[11] L.A. Byers, L. Diao, J. Wang, P. Saintigny, L. Girard, M. Peyton, I.I. Wistuba, K.R. Coombes, J.D. Minna, J.V. Heymach, An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance, Clin. Cancer Res. 19 (1) (2013) 279–290, http://dx.doi.org/10.1158/1078-0432.CCR-12-1558.
[12] H. Uramoto, T. Iwata, T. Onitsuka, H. Shimokawa, T. Hanagiri, T. Oyama, Epithelial-mesenchymal transition in EGFR-TKI acquired resistant lung adenocarcinoma, Anticancer Res. 30 (2010) 2513–2517.
[13] Y. Sesumi, K. Suda, H. Mizuuchi, Y. Kobayashi, K. Sato, M. Chiba, M. Shimoji, T. Mitsudomi, Effect of dasatinib on EMT-mediated -mechanism of resistance against EGFR inhibitors in lung cancer cells, Lung Cancer 104 (2017 Feb) 85–90, http://dx.doi.org/10.1016/j.lungcan.
[14] C.M. Della Corte, U. Malapelle, E. Vigliar, F. Pepe, G. Troncone, V. Ciaramella, T. Troiani, E. Martinelli, V. Belli, F. Ciardiello, F. Morgillo, Efficacy of continuous EGFR-inhibition and role of Hedgehog in EGFR acquired resistance in human lung cancer cells with activating mutation of EGFR, Oncotarget 8 (14) (2017) 23020–23032, http://dx.doi.org/10.18632/oncotarget.15479.
[15] K. Suda, P.A. Bunn, C.J. Rivard, T. Mitsudomi, F.R. Hirsch, Primary double-strike therapy for cancers to overcome EGFR kinase inhibitor resistance: proposal from the bench, J. Thorac. Oncol. 12 (1) (2017 Jan) 27–35, http://dx.doi.org/10.1016/j.jtho.2016.09.003.
[16] Y. Seki, Y. Fujiwara, T. Kohno, E. Takai, K. Sunami, Y. Goto, H. Horinouchi, N. Yamamoto, K. Kuwano, Y. Ohe, Picoliter-droplet digital polymerase chain reaction-based analysis of cell-free plasma DNA to assess EGFR mutations in lung adenocarcinoma that confer resistance to tyrosine-kinase inhibitors, Oncologist 21 (2) (2016 Feb) 156–164, http://dx.doi.org/10.1634/theoncologist.2015-0288.
[17] J. Ni, L. Weng, Y. Liu, Z. Sun, C. Bai, Y. Wang, Dynamic monitoring of EGFR mutations in circulating cell-free DNA for EGFR-mutant metastatic patients with lung cancer: early detection of drug resistance and prognostic significance, Oncol. Lett. 13 (6) (2017) 4549–4557, http://dx.doi.org/10.3892/ol.2017.6022.
[18] M. Soucheray, M. Capelletti, I. Pulido, Y. Kuang, C.P. Paweletz, J.H. Becker, E. Kikuchi, C. Xu, T.B. Patel, F. Al-Shahrour, J. Carretero, K.K. Wong, T. Shimamura, Intratumoral heterogeneity in EGFR mutant NSCLC results in divergent resistance mechanisms in response to EGFR tyrosine kinase inhibition, Cancer Res. 75 (20) (2015) 4372–4383, http://dx.doi.org/10.1158/0008-5472.CAN-15-0377.
[19] E. Ichihara, C.M. Lovly, Shades of T790M: intratumor heterogeneity in EGFR -mutant lung cancer, Cancer Discov. 5 (7) (2015) 694–696, http://dx.doi.org/10.1158/2159-82 90.CD-15-0616.
[20] R.A. Burrell, C. Swanton, Tumour heterogeneity and the evolution of polyclonal drug resistance, Mol. Oncol. 8 (6) (2014) 1095–1111, http://dx.doi.org/10.1016/j.molonc.