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

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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 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.

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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). Vimentin 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.)
2. Case report

A 52-year-old woman was diagnosed with moderate differentiated adenocarcinoma (T1NxM1a with visceral pleural nodules) harboring an \(\text{EGFR} \text{ exon } 19\) 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. 1A). 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. 1C, Table 1). E-cadherin were positive in the differentiated cancerous tissue but attenuated 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 recurrent tumor specimens, tumor tissue in a clot-like expectoration of the patient were collected two months before the patient died. Surprisingly, the recurrent 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 recurrent 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 lesion. As expectedly, the secondary \(\text{T790M}\) mutation in exon 20 was detected in the recurred lesions (Table 1).

3. Discussion & conclusion

The most prevalent mechanisms of acquired \(\text{EGFR}\)-TKI resistance are the \(\text{EGFR} \text{ T790M}\) mutation, \(\text{PIK3CA}\) mutation, \(\text{MET}\) 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 \(\text{T790M}\) mutation in the recurred lesions may explain the \(\text{EGFR}\)-TKI resistance reasonably because of the lacking of \(\text{T790M}\) 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 \(\text{T790M}\) are unknown, some study showed that EMT plays roles in resistance to \(\text{EGFR}\)-TKI but independent of \(\text{T790M}\) mutation [12]. Generally, secondary \(\text{T790M}\) mutation and EMT phenomenon are considered separately in acquisition of \(\text{EGFR}\)-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 \(\text{T790M}\) 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 been 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 \(\text{EGFR}\)-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 \(\text{T790M}\) 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.

Conflict of interests

The authors report no conflict of interests.

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Table 1

| Genetic mutation and EMT markers | 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              | (−)            |
| E-Cadherin                       | (++)            | (−)             | (+)            |
| Vimentin                         | (++)            | (+)             | (+)            |
| TTF-1                            | (++)            | (−)             | (−)            |

NA: not available.
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