Histological evolution from primary lung adenocarcinoma harboring EGFR mutation to high-grade neuroendocrine carcinoma

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Abstract

Background: Although patients with EGFR mutated lung adenocarcinoma benefit greatly from tyrosine kinase inhibitors (TKIs), they inevitably develop acquired resistance after an average of 10–14 months of continuous treatment.

Methods: We retrospectively analyzed the clinical and histopathological data of eight patients with primary lung adenocarcinoma harboring EGFR mutations that transformed into high-grade neuroendocrine carcinoma after TKI therapy. Morphology scanning for neuroendocrine differentiation and immunohistochemistry for neuroendocrine markers CD56, chromogranin, and synaptophysin were performed on primary adenocarcinoma tissues and repeated biopsies. Mutations of EGFR exons 19–21 were reexamined using the amplification refractory mutation system.

Results: The carcinoma in seven patients transformed to small cell lung carcinoma; two of these patients enrolled in theAZD9291 study after acquiring a T790M missense mutation. The carcinoma in one patient transformed to large cell neuroendocrine carcinoma. None of the eight primary tumors exhibited neuroendocrine morphologic features and only one surgical specimen displayed a weak stain for neuroendocrine marker synaptophysin. Drug resistant high-grade neuroendocrine carcinomas retained their initial activating EGFR mutations.

Conclusions: Lung adenocarcinoma in eight patients transformed into high-grade neuroendocrine carcinoma and retained the original activating EGFR mutations after targeted therapy by TKIs. Furthermore, the prognosis of the transformed carcinoma was worse than the original primary genetic and morphologic type.

Introduction

Tyrosine kinase inhibitors (TKIs) are applied as first-line treatment in patients with primary lung adenocarcinoma harboring active EGFR mutations.1,2 Although more patients benefit from TKI therapy, acquired drug resistance is inevitable after a median of approximately 10–14 months of treatment.3 To improve survival, the mechanism of drug resistance and clinical coping strategies need to be firmly established.

Acquired T790M is the primary mechanism of resistance to first-generation EGFR-TKIs. About half of the patients administered gefitinib or erlotinib develop varying degrees of drug resistance.4,5 Relevant research has shown that patients who acquired T790M could further benefit from third-generation TKIs after treatment failure with previous TKIs.6-7 Other mechanisms or signaling pathways can affect this process, such as MET gene amplification, second point mutations, PIK3CA or BRAF mutations, epithelial-mesenchymal transition, and high-grade neuroendocrine tumor transformation to large cell neuroendocrine carcinoma (LCNEC), small cell lung carcinoma (SCLC), and their corresponding combined type.8-11

Histological transformation from non-small cell lung carcinoma (NSCLC) to SCLC or LCNEC has been reported in a subset of resistant patients, but the morphology and molecular transformation process is still obscure.12-14 To study this evolution, we undertook comprehensive EGFR status and histomorphological analysis of eight patients with primary lung adenocarcinoma harboring EGFR mutations that transformed into high-grade neuroendocrine...
carcinoma after TKI therapy. \textit{EGFR} status and neuroendocrine markers were re-detected in all initial specimens and multiple points of biopsies.

**Methods**

**Patients and tissues**

The eight \textit{EGFR}-mutated lung adenocarcinoma patients included in this study were hospitalized at the Department of Medical Oncology or Chest Surgery from April 2011 to May 2017. All patients had primary lung adenocarcinoma and \textit{EGFR} activating mutations. No chemotherapy, radiotherapy, or traditional Chinese medicine was administered before biopsy or surgery. Pulmonary lobectomy surgery was performed in three patients. Five primary tumors were diagnosed by endobronchial ultrasound with transbronchial needle aspiration or metastatic lymph node, transbronchial, or fine needle lung biopsy. All treatment options were performed in our hospital with the exception of one patient who received first-line chemotherapy at a local hospital. The electronic medical record system was retrospectively reviewed to obtain all imageological examinations and clinical information. We obtained ample repeat biopsy samples from all patients after the failure of maintenance treatment. Tissue samples for morphological evaluation and molecular analysis included lobectomy specimens, lymph node cellblocks, supraclavicular lymph node biopsies, and fine needle biopsies of lung lesions. The clinicopathological features of the eight patients are summarized in Table 1.

**Histological identification**

Three lobectomy specimens were confirmed by pathological diagnosis according to the 2015 World Health Organization classification of tumors of the lung. After thorough histologic examination by two pathologists with at least 10 years of diagnostic experience, evidence of neuroendocrine morphology was excluded. Five patients were diagnosed by cytology or biopsy. Three markers for neuroendocrine tumors, including synaptophysin, chromogranin, and CD56, were performed to define and recognize SCLC/LCNEC on repeated biopsies. Ki-67 was used to evaluate the proliferation index. Representative tissue sections (5 \textmu m) were cut from the paraffin-embedded tissue blocks. Immunohistochemistry was performed using standard protocols.

| Patient ID# | Gender | Age (years) | Smoking history | Initial tissue | Histology | Stage | EGFR† |
|-------------|--------|-------------|-----------------|---------------|-----------|-------|-------|
| 1           | Female | 55          | No              | 4R lymph node (EBUS-TBNA) | Ad        | IV cT1bN2M1 | Exon 19 del |
| 2           | Female | 45          | No              | Right supraclavicular lymph node biopsy | Ad        | IV cT2N3M1 | Exon 19 del |
| 3           | Female | 50          | No              | Transbronchial lung biopsy | Ad        | IV cT2bN0M1 | Exon 19 del |
| 4           | Male   | 65          | Yes             | Pulmonary resection | Ad        | IB pT2aN0M0 | Exon 19 del |
| 5           | Female | 53          | No              | Fine needle lung biopsy | Ad        | IV cT4N3M1c | Exon 19 del |
| 6           | Male   | 47          | No              | Fine needle lung biopsy | Ad        | IVcT2aN1M1 | L858R |
| 7           | Male   | 71          | No              | Pulmonary resection | Ad        | II A pT2bN0M0 | L858R |
| 8           | Male   | 52          | Yes             | Pulmonary resection | Ad        | III A pT2N2M0 | L858R |

†EGFR mutation in initial tissue. Ad, adenocarcinoma; EBUS-TBNA, endobronchial ultrasound-transbronchial needle aspiration.

**Figure 1** Chest computed tomography imaging of lung adenocarcinoma patients harboring \textit{EGFR} mutations. 1–8 shows the primary lung tumor in the eight patients.
from formalin-fixed, paraffin-embedded specimens were used to perform immunohistochemical staining using an auto-stainer GI100 (DAKO OMNIS/Agilent, Santa Clara, CA, USA) following the manufacturer’s instructions.

Detection of EGFR mutation

Each specimen was reviewed and stained with hematoxylin and eosin to select representative areas of tumor lesions to ensure at least 200 tumor cells were preserved in paraffin sections for EGFR detection. Tumor DNA extracted from formalin-fixed, paraffin-embedded tissue and cellblocks was used to detect mutation of EGFR exons 19–21 using direct DNA sequencing (frequency = 4) or the amplification refractory mutation system (frequency = 14) following the manufacturer’s instructions. The amplification refractory mutation system has been used as standard for clinical analysis in our institute since December 2013. All slides and molecular detection results were confirmed by two of the authors.

Detection of neuroendocrine differentiation in primary tumor tissues

Neuroendocrine markers CD56, chromogranin, and synaptophysin were detected in primary adenocarcinoma tissues to exclude the possibility of the presence neuroendocrine components in poorly differentiated regions.
Results

Histological evaluation

Chest computed tomography (CT) imaging of the primary tumor and corresponding histomorphology are shown in Figures 1 and 2. Seven patients transformed to SCLC and one transformed to LCNEC. Six patients were diagnosed with high-grade neuroendocrine carcinoma in their second biopsy and two patients in their third biopsy. The second biopsies of the first two patients confirmed the original diagnosis of adenocarcinoma by fine needle biopsy of lung and 4R lymph node cellblock, respectively. A diagnosis of SCLC was based on cellblocks or biopsy obtained from a new lung lesion or cervical lymph node biopsy, while LCNEC was proven histologically by bronchoscopy brush cell smears and fine needle lung biopsy of the relapsed lesion (see Fig 3).

Treatment duration

Patients 1–6 were administered gefitinib or erlotinib as first-line treatment, while patients 7 and 8 received vinorelbine-carboplatin (NC) chemotherapy. All patients responded to TKIs or chemotherapy on different levels. A second biopsy was performed on two patients at the time of disease progression, and they enrolled in the AZD9291 study after acquiring a T790M missense mutation. Four patients chose gefitinib or icotinib as maintenance therapy. Patient 5 was administered alimta-carboplatin and avastin, while patient 8 received chemoradiotherapy for further treatment. Six patients with transformed-small cell lung carcinoma (t-SCLC) underwent further etoposide-carboplatin (EC) chemotherapy. The transformed-large cell neuroendocrine carcinoma (t-LCNEC) patient ceased medical treatment and died after five months. Patient 1 achieved a good response to EC treatment and underwent six courses, while patient 2 continued the original strategy after the remarkable effects of AZD9291. The other five patients had relatively indolent disease and received 3–5 courses of typical chemotherapy to treat SCLC. The treatment methods and molecular transformation process from primary histology to high-grade neuroendocrine carcinoma are listed in Table 2. The imaging dates and history of anticancer therapy for typical patients are shown in Figure 4 and Table 3.

EGFR status

The primary tumor tissue of the first five patients had EGFR exon 19 deletions, while the remaining three patients had an L858R mutation on exon 21. First-generation EGFR-TKI treatment failed in patients 1 and 2 after
18 and 40 months, respectively, after they acquired T790M missense mutations. Their second biopsies indicated both 19del and T790M. After 10–13 months of oral AZD929, their t-SCLC tissues retained 19del, but T790M could no longer be detected. All drug-resistant transformed high-grade neuroendocrine carcinoma tissues retained their initial activating \(\text{EGFR}\) mutations.

### Analysis of neuroendocrine differentiation

None of the eight primary tumor specimens exhibited neuroendocrine morphologic features. Only one lobectomy specimen showed a weak positive stain for synaptophysin, but was negative for CD56 and chromogranin (Fig 5). At least one neuroendocrine marker (CD56, chromogranin, or synaptophysin) detected strong membrane and/or cytoplasm positive in t-SCLC and t-LCNEC tissues.

### Discussion

We examined \(\text{EGFR}\) status and performed histological analyses on eight primary lung adenocarcinoma patients. All of the tumor specimens underwent histopathological and immunohistochemical assay, and significant alterations were observed. Neuroendocrine morphologic features were not recognized in every primary tumor tissue. A weak positive stain for the neuroendocrine marker synaptophysin was found in only one lobectomy primary tumor. Carcinoma in seven patients transformed to SCLC and in one patient to LCNEC. Two patients acquired \(\text{EGFR}\) (T790M) mutations after 11–40 months of gefitinib therapy. Although they
enrolled in the AZD9291 study, they developed drug resistance after 7–13 months of third-generation TKI treatment and their carcinoma eventually transformed into SCLC.

These eight patients responded to the typical chemotherapy used to treat SCLC and LCNEC at different levels. However, despite their initial treatment strategy, all patients acquired resistance to EGFR inhibitors through histological transformation, and the duration from treatment to disease progression was about 11–52 months (median 18). The third generation TKIs could block the T790M mutation and maintain 7–13 months remission. Furthermore, subsequent treatment for SCLC and LCNEC was not encouraging compared to corresponding treatment for primary high-grade neuroendocrine tumors.

Throughout the treatment process of these patients, we found that TKIs as the first-line strategy led to better survival than traditional chemotherapy. Most importantly, the overall survival rate seemed to be more dependent on the sensitivity of chemotherapy after the transformation into high-grade neuroendocrine carcinoma. However, overall survival was not optimized, as three patients eventually died of converted high-grade neuroendocrine carcinoma after 4–12 months.

The morphological transformation of these eight patients was closely related to TKI treatment; however, little is known about this exact process. It is difficult to control rapid progressive disease after transformation. Although typical chemotherapy for secondary high-grade tumors is less sensitive, there is currently no alternative method to treat secondary neuroendocrine carcinoma. Moreover, because of the limitations and delays with biopsies, we were unable to accurately assess whether the adenocarcinoma tissue had completely transformed to SCLC or had only partially transformed, and thus was defined as combined neuroendocrine tumor.

Some scholars consider this transformed high-grade neuroendocrine tumor as a special subtype. High-grade endocrine tumors and transformed high-grade endocrine tumors share similar morphology and protein levels, but have different genetic alterations. Recently, Matthew et al. and Mariko et al. suggested that this subset of resistant adenocarcinomas ultimately adopt many of the molecular and phenotypic characteristics of classical SCLC or

Figure 5 Neuroendocrine expression in the primary tumor. (a) Hematoxylin and eosin staining of surgical lobectomy in patient 7. (b, c) TTF-1 and NapsinA were used to define primary lung adenocarcinoma. (d) A weak positive stain of synaptophysin was detected as a typical acinar component. (e, f) CD56 and chromogranin were negative.
LCNCC, such as retinoblastoma loss, greater sensitivity to BCL2 family inhibition, increased neuroendocrine markers and decreased EGFR expression.17,18 Yet we still face huge challenges in the clinical setting. What is the proper time to obtain a second biopsy and what is the appropriate time to send samples for genetic testing? When the primary tumor progresses, how do we choose a practical countermeasure between targeted therapy and other chemotherapeutic strategies?

In our reported cases, histological transformation from primary adenocarcinoma to high-grade neuroendocrine carcinoma was closely related to TKI treatment. All drug-resistant SCLC or LCNCC tumors retained their initial activating EGFR mutations and had particular clinical features different from those of primary lung neuroendocrine tumors. Although the first-line treatment and mutation sites were different, these primary lung adenocarcinomas have revolutionized our understanding of resistance mechanisms. The process of drug-resistance and clinical treatment strategies for transformed high-grade neuroendocrine carcinomas require further investigation.

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Disclosure

No authors report any conflict of interest.

References

1 Sharma SV, Bell DW, Settleman J et al. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 2007; 7: 169–81.
2 Zhou C, Wu YL, Chen G et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011; 12: 735–42.
3 Mok TS, Wu YL, Thongprasert S et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. New Engl J Med 2009; 361: 947–57.
4 Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: Learning from lung cancer. Nat Rev Clin Oncol 2014; 11: 473–81.
5 Sequist LV, Waltman BA, Dias-Santagata D et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011; 3 (75): 75ra26.
6 Jänne PA, Yang JC, Kim DW et al. AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med 2015; 372: 1689–99.
7 Sequist LV, Rolfe V, Allen AR et al. Rociletinib in EGFR-mutated non-small-cell lung cancer. N Engl J Med 2015; 373: 578–9.
8 Ohashi K, Sequist LV, Arcila ME et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A 2012; 109: e2127–33.
9 Toyooka S, Date H, Uchida A, Kiura K, Takata M. The epidermal growth factor receptor D761Y mutation and effect of tyrosine kinase inhibitor. Clin Cancer Res 2007; 13: 3431.
10 Levin PA, Mayer M, Hoskin S, Sailors J, Oliver DH, Gerber DE. Histologic transformation from adenocarcinoma to squamous cell carcinoma as a mechanism of resistance to EGFR inhibitor. J Thorac Oncol 2015; 10: e86–8.
11 Furugen M, Uechi K, Hirai J et al. An autopsy case of two distinct, acquired drug resistance mechanisms in epidermal growth factor receptor-mutant lung adenocarcinoma: Small cell carcinoma transformation and epidermal growth factor receptor T790M mutation. Intern Med 2015; 54: 2491–6.
12 Jiang SY, Zhao J, Wang MZ et al. Small-cell lung cancer transformation in patients with pulmonary adenocarcinoma: A case report and review of literature. Medicine (Baltimore) 2016; 95: e2752.
13 Kim WJ, Kim S, Choi H et al. Histological transformation from non-small to small cell lung carcinoma after treatment with epidermal growth factor receptor-tyrosine kinase inhibitor. Thorac Cancer 2015; 6: 800–4.
14 Yu HA, Arcila ME, Rekhtman N et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res 2013; 19: 2240–7.
15 Norkowski E, Ghigna MR, Lacroix L et al. Small-cell carcinoma in the setting of pulmonary adenocarcinoma: New insights in the era of molecular pathology. J Thorac Oncol 2013; 8: 1265–71.
16 Belchis DA, Tseng LH, Gniadek T et al. Heterogeneity of resistance mutations detectable by next-generation sequencing in TKI-treated lung adenocarcinoma. Oncotarget 2016; 7: 45237–48.
17 Niederst MJ, Sequist LV, Poirier JT et al. RB loss in resistant EGFR mutant lung adenocarcinomas that transform to small-cell lung cancer. Nat Commun 2011; 6: 6377.
18 Kogo M, Shimizu R, Uehara K et al. Transformation to large cell neuroendocrine carcinoma as acquired resistance mechanism of EGFR tyrosine kinase inhibitor. Lung Cancer 2015; 90: 364–8.