Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

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

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Keywords
EGFR; lung adenocarcinoma; SCLC; T790M; TKI.

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Received: 22 August 2017;
Accepted: 27 September 2017.

doi: 10.1111/1759-7714.12549

Thoracic Cancer **9** (2018) 129–135

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

Table 1 Clinicopathological features of eight primary lung adenocarcinoma patients

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	IVcT2N3M1	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	IVcT4N3M1c	Exon 19 del
6	Male	47	No	Fine needle lung biopsy	Ad	IVcT2aN1M1	L858R
7	Male	71	No	Pulmonary resection	Ad	IIA pT2bN0M0	L858R
8	Male	52	Yes	Pulmonary resection	Ad	IIIApT2N2MO	L858R

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

carcinoma after TKI therapy. *EGFR* status and neuroendocrine markers were re-detected in all initial specimens and multiple points of biopsies.

Methods

Patients and tissues

The eight *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 *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 cell-blocks, 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 (5μm)

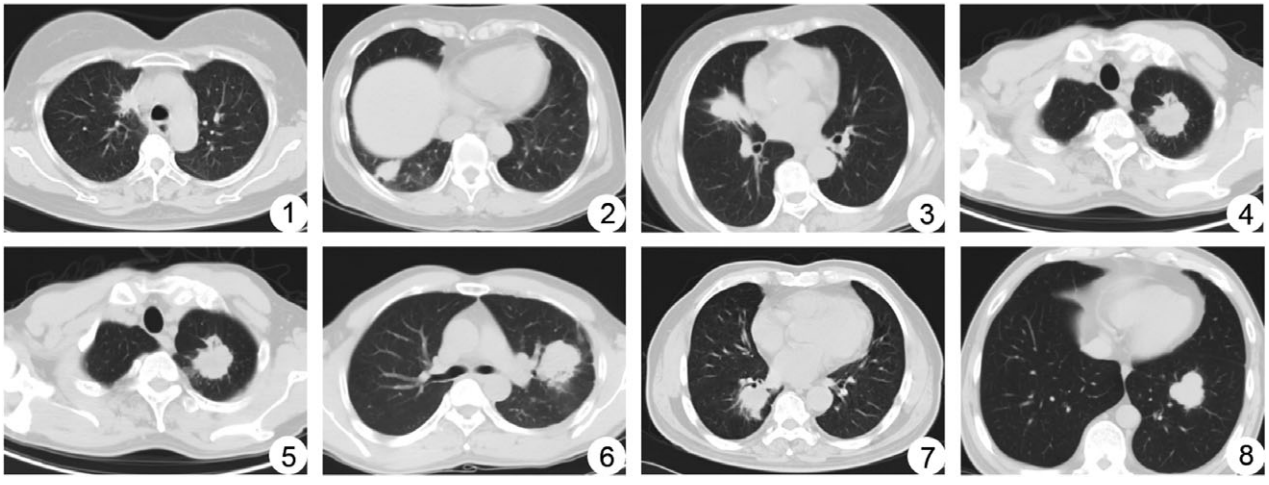

Figure 1 Chest computed tomography imaging of lung adenocarcinoma patients harboring *EGFR* mutations. **1–8** shows the primary lung tumor in the eight patients.

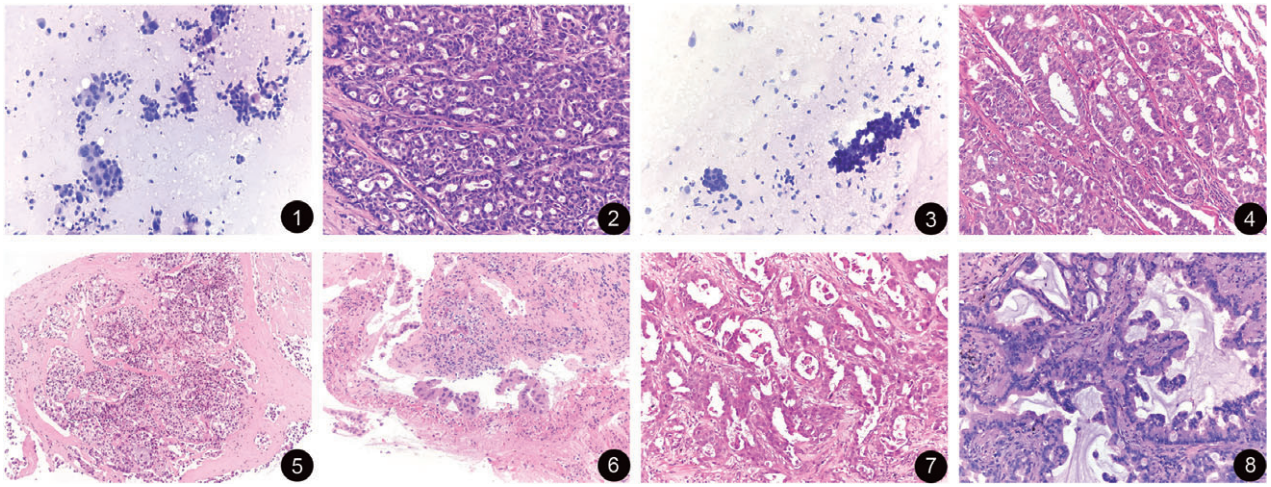

Figure 2 Cytological and histopathological assessment in eight patients: (**1**) 4R lymph node suggested an adenocarcinoma tumor; (**2**) biopsy of right supraclavicular lymph node showed metastatic adenocarcinoma characterized a cribriform growth pattern; (**3**) transbronchial lung biopsy of the middle lobe of the right lung displayed cohesive adenocarcinoma cells and normal bronchial epithelium; (**5**) fine needle lung biopsy (FNLB)exhibited solid tumor growth infiltrating fibrous tissue; (**6**) FNLB presented a few clusters of adenocarcinoma cells; (**4** and **7**) acinar and papillary predominant invasive adenocarcinoma with areas mimicking a cribriform pattern demonstrated in pulmonary resection tumors; (**8**) this resected tumor showed papillary predominant adenocarcinoma with mucin production.

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.

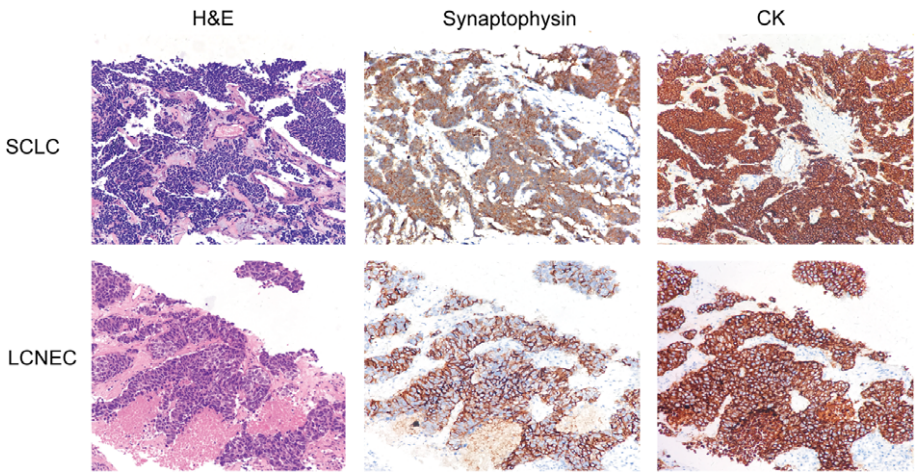

Figure 3 Diagnosis of transformed high-grade neuroendocrine carcinomas: hematoxylin and eosin (H&E 20×) stain displayed the classic histology of small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC). The final diagnosis was confirmed by immunostaining of CD56, pCK, and TTF-1.

Table 2 Treatment and molecular transformation process from primary histology to high-grade neuroendocrine carcinoma

Patient ID#	First-line treatment	Time to disease progression (months)	Second biopsy after disease progression	EGFR†	Subsequent treatment	Repeat biopsy	Time to Ad/SCLC or LCNEC transformation (months)	EGFR‡	Treatment for SCLC/LCNEC	Follow-up status (months)
1	Gefitinib	11	Pleural Biopsy	19del + T790M	AZD9291	FNLB SCLC	28	19del	6 courses of EC	Died (40)
2	Gefitinib	40	LSCLN	19del + T790M	AZD9291	4R LN SCLC	53	19del	AZD9291	Alive (58)
3	Gefitinib	52	—	—	Gefitinib	FNLB SCLC	58	19del	4EC + radiotherapy	Alive (66)
4	Gefitinib	18	—	—	Gefitinib	LSCLN SCLC	22	19del	2EC	Alive (31)
5	Erlotinib	14	—	—	AC + Avastin	FNLB SCLC	15	19del	AC + Avastin	Alive (18)
6	Gefitinib	14	—	—	Gefitinib	FNLB SCLC	20	L858R	5EC	Alive (25)
7	Chemotherapy	16	—	—	Icotinib	TBLB SCLC	45	L858R	1E	Died (49)
8	Chemotherapy	18	—	—	Chemoradiotherapy	FNLB LCNEC	55	L858R	None	Died (60)

†EGFR mutation in second biopsy. ‡EGFR mutation in small cell lung carcinoma (SCLC)/large cell neuroendocrine carcinoma (LCNEC) specimen. AC, alimta-carboplatin; Ad, adenocarcinoma; EC, etoposide-carboplatin; FNLB, fine needle lung biopsy; LSCLN, left supraclavicular lymph node; TBLB, transbronchial lung biopsy.

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

Table 3 Patient #1: History of anticancer therapy

Date	Disease progressing	Treatment	Cycles	Response
December 2013	—	Gefitinib	—	PR
December 2014	PD	Gefitinib + (tegafur, gimeracil and oteracil potassium)	—	SD
May 2015	Osseous metastasis and pleural effusion	Gefitinib + ibandronate + intrapleural injection cisplatin	1	PD
June 2015	—	Pemetrexed + carboplatin	2	PD
September 2015	T790M	AZD9291	—	NE
March 2016	SCLC harboring 19del+	Gefitinib + (tegafur, gimeracil and oteracil potassium)	—	PD
May 2016	—	VP16 + carboplatin	6	PD

NE, not evaluable; PD, progressive disease; PR, partial remission; SD, stable disease.

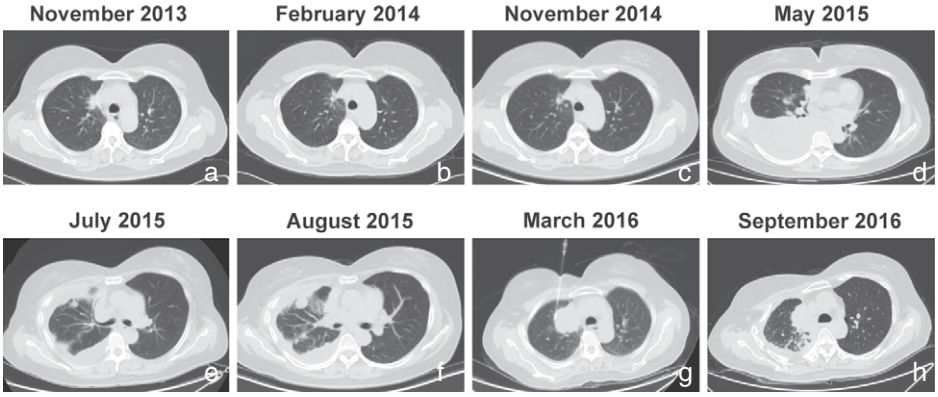

Figure 4 Imaging date and tissue access of representative case (patient 1). (**a**) Chest computed tomography (CT) showed a dense soft tissue mass in the upper right lung; transbronchial needle aspiration (TBNA) of the 4R lymph node suggested adenocarcinoma. (**b**) Five months after oral gefitinib administration, CT indicated stable disease. (**c**,**d**) CT indicated slow progressive disease (PD): a nodule of the upper right lung and paramediastinum with pleural effusion at 18 months. (**e**) Subsequent pleural biopsy and histological examination confirmed adenocarcinoma recurrence. (**f**) Gefitinib was ceased and AZD9291 treatment was commenced; CT indicated PD. (**g**) Fine needle lung biopsy was performed after 28 months and revealed transformation to small cell lung carcinoma. (**h**) After three courses of etoposide-carboplatin chemotherapy, a reduction in the tumor was not obvious.

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 *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 *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 *EGFR* (T790M) mutations after 11–40 months of gefitinib therapy. Although they

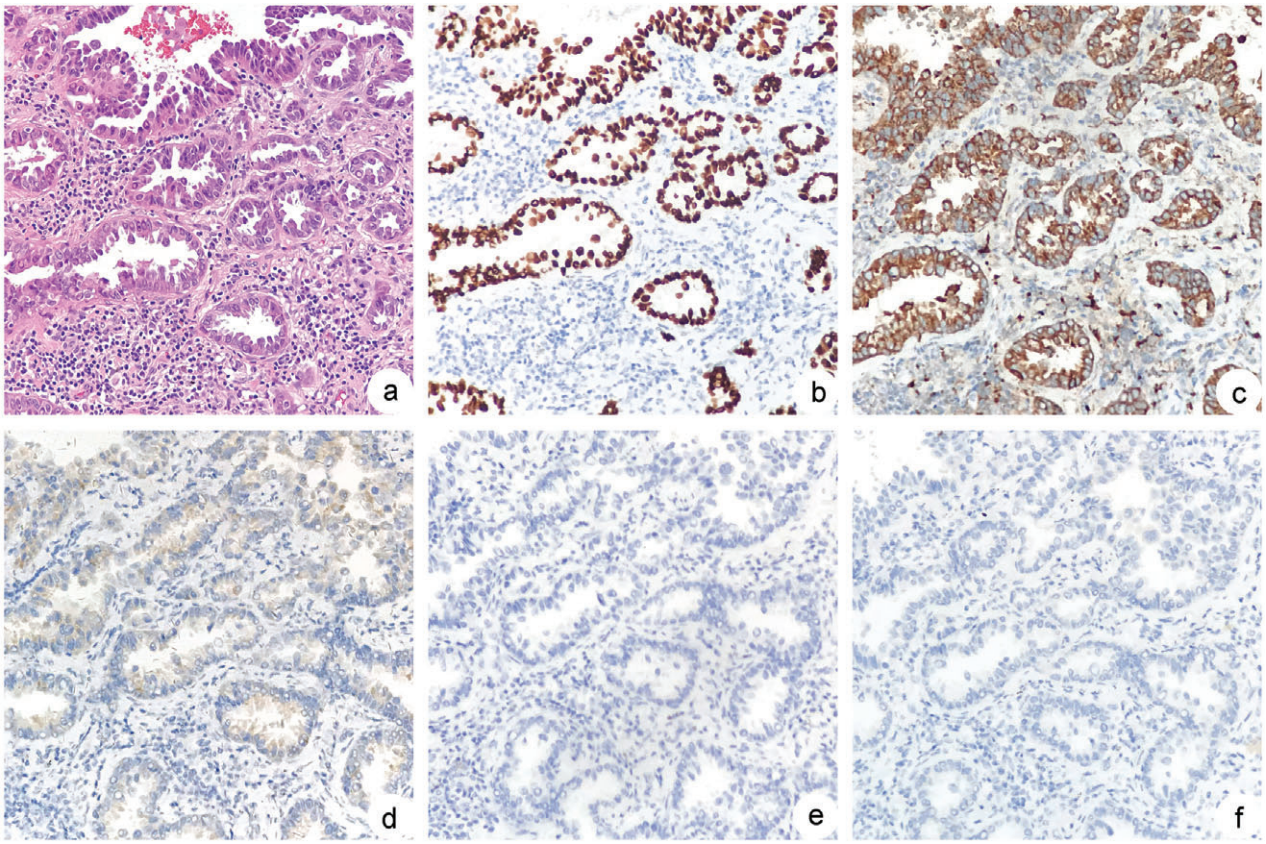

Figure 5 Neuroendocrine expression in the primary tumor. (**a**) Hematoxylin and eosin staining of surgical lobectomy in patient 7. (**b**,**c**) TTF-1 and NapsinA were used to define primary lung adenocarcinoma. (**d**) A weak positive stain of synaptophysin was detected as a typical acinar component. (**e**,**f**) CD56 and chromogranin were negative.

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.[15,16] 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

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.

Acknowledgment

We would like to acknowledge and thank our colleagues in the medical records and imaging departments.

Disclosure

No authors report any conflict of interest.

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