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Case Report

Response to EGFR-TKI in patients with gastrointestinal metastasis from primary lung adenocarcinoma: report of two cases

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Abstract: Lung cancer is the leading cause of cancer-related mortality worldwide. The most common sites of lung cancer metastasis are the lymph nodes, brain, liver, bones, and adrenal glands, whereas the gastrointestinal (GI) tract is rare. Here we report two cases of non-small cell lung cancer with GI metastases. Both patients were given epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) during treatment. The patient of case 1 died soon after being given icotinib for late-stage multiple organ failure. The patient of case 2 with wild-type EGFR achieved 13 months of progression-free survival after erlotinib therapy, and the most recent follow-up evaluation suggested a stable condition in which no lesions demonstrated progression. Through these two cases, we observed different clinical effects and prognosis after EGFR-TKI-based therapy for these patients, but the mechanism of action requires further elucidation.

Keywords: Non-small cell lung cancer, gastrointestinal metastasis, EGFR-TKI, EGFR mutation analysis, haplotype

Introduction

Lung cancer is the leading cause of cancer deaths worldwide [1]. At the time of diagnosis, approximately half of cases are non-resectable due to metastasis to various organs, including the brain, liver, bones, and adrenal glands. However, gastrointestinal (GI) tract metastasis is uncommon and usually neglected. Cases of lung cancer initially manifesting as GI tract metastasis are exceedingly rare according to a literature review, representing a diagnostic challenge and a late-stage disease sign [2]. As early as 1982, Antler et al reviewed the autopsy data of 423 cases of primary lung cancer over a 36-year period to evaluate the presence of GI metastasis. Fifty-eight cases (14%) were found, and the esophagus was the most commonly involved site [3]. Although GI metastasis is more common than previously thought from autopsy data [3, 4], symptomatic GI metastases are fairly rare in live patients because the lesions are frequently considered side effects of chemotherapy such as ulcers, enteritis, or colitis. Lung cancer with GI metastasis generally has poor prognosis.

The identification of activating mutations in EGF receptor-tyrosine kinase inhibitors (EGFR-TKI) has changed the standard approach for evaluating advanced non-small cell lung cancer (NSCLC) and established tumor genotyping in daily clinical practice [5]. Randomized phase III trials have demonstrated that the use of EGFR-TKI has led to impressive clinical responses in patients with NSCLC [6]. These clinical trials have shown that 75-80% of patients with lung cancer carrying EGFR mutations attained dramatic radiographic responses to TKI treatment [6]. In addition, progression-free survival (PFS) and overall survival (OS) were significantly better for patients with EGFR mutations than those with wild-type EGFR [6, 7]. Moreover, patients with EGFR mutations treated in the maintenance setting had a large benefit in term of PFS.

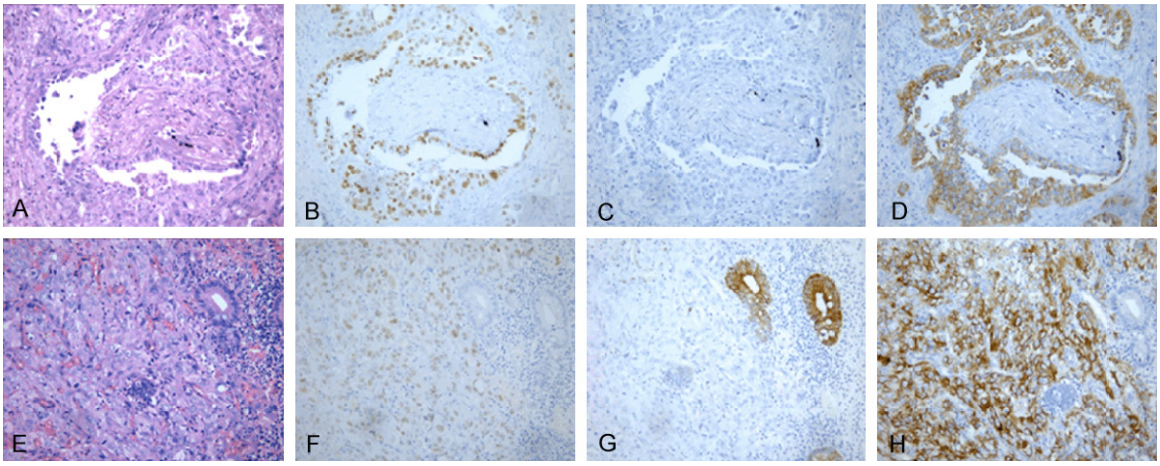

Figure 1. Hematoxylin and eosin (H&E) and immunohistochemical staining of the primary lung cancer and rectum metastatic tumor tissues in case 1. H&E staining showed the adenocarcinomous area of the primary lung cancer (A) was similar to that of a rectal metastasis (E). Immunohistochemical staining were also identical in primary lung cancer tissues and rectal metastatic tumor tissues, which tested positive for thyroid transcription factor-1 (B and F) and cytokeratin (CK)-7 (D and H) but negative for CK-20 (C and G). The upper panels show the primary lung cancer, while the lower panels show the rectal metastasis (×400).

In this report, we describe the clinicopathological and immunohistochemical features and therapeutic processes of two cases of primary lung cancer with GI metastases.

Case presentation

Case 1

A 62-year-old man presented with cough and hemoptysis for several months was referred to a hospital in March 2013. A chest computed tomography (CT) scan showed a mass in the right upper lobe. A routine CT examination found no distant metastases. Subsequently, he underwent resection of the right upper lobe and excision of the regional lymph nodes on April 7, 2013. The pathology was infiltrating lung adenocarcinoma.

The patient received four cycles of adjuvant chemotherapy with cisplatin and pemetrexed. Two months later after the last adjuvant chemotherapy cycle, the hemoptysis resumed, and a chest CT scan (November 22, 2013) showed small nodules within both lungs, enlargement of the right hilar lymph node, and focal dust emphysema associated with pulmonary bullae formation. However, the sputum cytology and bronchoscope examination revealed no cancer cells. Another two months later, right lower abdominal pain and hematochezia appeared. A diagnostic colonoscopy revealed a polypoid mass in the rectum. The patient underwent endoscopic ligation snare resection. The cytomorphology showed adenoma, and the immunohistochemical features (thyroid transcription factor-1 [TTF-1]+, cytokeratin [CK]-7+, CK-20-) confirmed that the rectal adenoma was a metastatic lesion from lung adenocarcinoma (**Figure 1**).

The patient was referred to our hospital, and a general CT scan revealed metastases into the bilateral lungs, pleura, right parietal lobe, posterior horn of the lateral ventricles, and left adrenal metastases for which he underwent lung and brain radiotherapy and two cycles of chemotherapy with docetaxel and carboplatin. A follow-up CT showed increased lung and pleura masses and a new mass in the left lobe of the liver. His disease progressed and his physical condition deteriorated, preventing further chemoradiotherapy treatments. After communicating with his family, the patient was tentatively given icotinib, but no clinical response was observed. He passed away approximately 13 months after his NSCLC diagnosis.

Case 2

A 44-year-old never-smoker woman was admitted with a 2-week history of paroxysmal periumbilical pain and radiating lumbar and back pain that worsened after eating. An abdominal CT

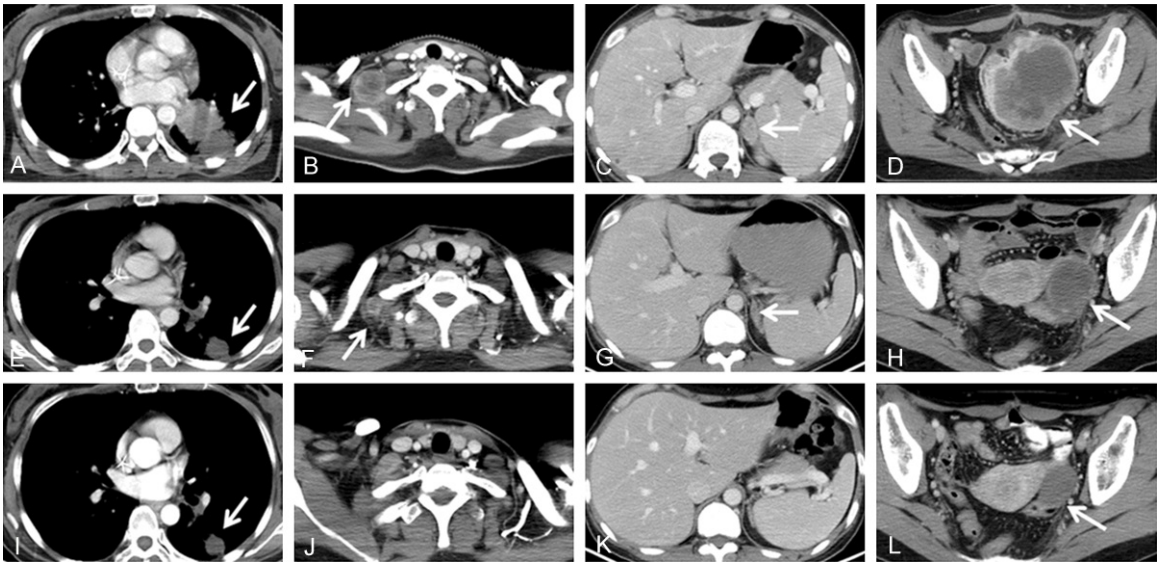

Figure 2. Computed tomography (CT) scan of the masses before and after Tarceva administration in case 2. CT scan showing the left lung hilar mass and the lower left lung mass (A, E, I), right cervical roots with swollen lymph nodes (B, F, J), left adrenal mass (C, G, K), and left accessory mass (D, H, L). (A-D) Show images taken before Tarceva treatment, while (E-G), and (H) show images taken 1 month after Tarceva administration. (I-L) Show images taken 2 months after Tarceva treatment. The tumoral mass in the right cervical root (J) and the left adrenal gland (K) narrowed obviously and could not be seen 2 months after Tarceva administration.

scan (September 18, 2013) showed a mass in the left center of the small intestine. A chest x-ray showed a fuzzy shadow in the lower left lung hilum. Chest CT showed a mass behind the lower left lung hilum suggestive of lung cancer and an elliptic nodule in the lower left lobe. Despite fasting and gastrointestinal decompression, her abdominal pain worsened.

Contrast-enhanced CT scan showed a small bowel obstruction of the lower left abdomen and a terminal ileum intussusception for which she underwent an emergent exploratory laparotomy on September 26, 2013. She was found to have a 2 × 2 cm hard polypoid mass in the upper jejunum and a tumor with intussusception in the ileum. The pathology was adenocarcinoma. According to CT and immunohistochemical results, the intestinal tumor was possibly a metastasis from lung cancer. She then began to cough and developed hemoptysis for which she came to our hospital. The pathological results of a needle biopsy from the lung mass revealed poorly differentiated primary lung adenocarcinoma. Immunohistochemical staining revealed that tumor cells from the small bowel and lung mass were positive for CK-7 and TTF but negative for CK-20, indicating that the metastatic adenocarcinoma originated from the lung cancer (same as case 1). We assessed formalin-fixed, paraffin-embedded biopsy specimens from the lung mass for EGFR mutation (exons 18-21) using direct sequencing (ABI 377) but found no mutation. A cerebral CT scan demonstrated a unique metastasis in the left frontal lobe, while abdominal CT demonstrated a mass on the left side of the uterine attachment.

The patient initially received two cycles of carboplatin and pemetrexed chemotherapy. A follow-up chest and abdominal CT scan (December 11, 2013) demonstrated a progression in the primary lung lesion and lymph nodes, reduction in the left frontal lobe, and new metastasis in the temporal lobe. Thus, the regimen was changed to chemoradiotherapy with paclitaxel and nedaplatin and whole-brain radiotherapy. During the radiotherapy, she developed abdominal pain and vomiting. An abdominal CT scan revealed a mass in the small intestine and an incomplete intestinal obstruction. She subsequently underwent laparoscopic exploration, small intestinal tumor resection, and enterolysis on January 28, 2014, and the pathology was consistent with primary lung cancer. According to her previous treatment and efficacy, she was given lung radiotherapy and we

attempted to give her erlotinib in March, 2014. Unbelievably, 1 month later, a follow-up CT scan demonstrated a good partial response to erlotinib. All of the target lesions had narrowed significantly (**Figure 2**). Two months later, the primary lung lesion and multiple metastases reduced further. She is presently alive 18 months after the diagnosis and has been progression free for 13 months after erlotinib administration. The most recent follow-up evaluation suggested a stable condition.

Discussion

In lung cancer, the brain, liver, adrenal glands, and bone are the most likely sites of distant metastases. GI metastases are considered most unusual. Our two cases of NSCLC with GI metastasis are a rare occurrence in our institution. Lung cancer with GI metastasis reportedly has poor prognosis with a mean survival of only 4-8 weeks [8, 9]. McNeill et al found that all lung cancer patients with small bowel metastases in their series had at least one other metastatic site and that the average was 4.8 sites [10]. In our report, in addition to GI metastasis, they both had multiple other metastases, including to the brain, lymph nodes, and adrenal glands. The patient in case 1 here experienced rapid progression of the lung cancer and had atypical abdominal symptoms. He died soon after diagnosis of the rapidly advanced disease. In contrast, the primary tumor in case 2 was diagnosed after a small bowel metastasis resection, and that patient's clinical symptoms at presentation were consistent with an acute intestinal obstruction.

It is difficult to diagnose the origin of a gastrointestinal tumor because lung cancer involving the gastrointestinal tract has no peculiar features, meaning that it usually mimics a primary gastrointestinal tumor. Thus, immunohistochemical staining is the only way to identify metastatic tumors to the gastrointestinal tract, while immunostaining with TTF-1, CDX2, CK7, and CK20 is also helpful for distinguishing primary gastrointestinal carcinoma from metastasis of lung carcinoma [11]. Lung carcinomas usually have the CK7+/CK20- immunoprofile, whereas intestinal carcinomas are generally believed to possess the opposite CK7-/CK20+ immunophenotype [11]. Besides, TTF-1 is only expressed in lung and thyroid malignancies. In our study, both patients were CK7+, CK20-, and TTF-1+.

EGFR-TKI is the standard treatment in patients with advanced NSCLC harboring active EGFR mutations since it has demonstrated improved response rates, PFS, and quality of life compared with upfront chemotherapy [6]. Thus, EGFR mutation analysis is significant for the treatment decision-making process for predicting response. However, some studies have reported survival benefits in patients with NSCLC and wild-type EGFR upon erlotinib treatment [12].

In the present report, the histological type of the two patients was adenocarcinoma. Both received EGFR tyrosine kinase-directed therapy during the disease course. In case 1, we could not obtain samples, so neither the primary tumors nor the metastatic lesions were subjected to EGFR mutation testing. The patient in case 1 received EGFR-TKI therapy in the late disease course but died of cancer soon thereafter. One can only speculate that if the EGFR mutation was identified earlier in his disease course, there may have been one more treatment option. The patient in case 2 had no detected EGFR mutations but showed a good partial response after EGFR-TKI therapy.

The clinical practice guidelines recommend that EGFR molecular testing could be used to select patients for EGFR-targeted TKI therapy and the patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics alone. However, there is still no gold standard available to define the optimal testing method and sample type for detecting EGFR mutations. There are many methods available to identify EGFR mutations, including direct sequencing, real-time polymerase chain reaction, and DNA microarray. Among them, direct sequencing is widely used in clinical practice. In our study, the needle biopsy from the second patient was detected using direct sequencing to analyze the EGFR mutation information. However, direct sequencing is time-consuming and insensitive when viable tumor cells constitute < 20% of a sample [13]. Biopsy samples of NSCLC are usually not amenable to multiple molecular tests. Yi S et al suggest that the method used should be chosen based on sample type [14]. They found that direct sequencing was recommended when mutations were identified in surgical resections, while the use of an amplification-refractory mutation system was recommended for

bronchoscopic biopsies and cytological samples. Furthermore, false-positives and -negatives should not be neglected. The sensitivity and specificity of direct sequencing can influence the detection results and affect clinical decision-making [15, 16]. Thus, to some extent, re-examination of EGFR gene status using different methods or samples should be considered to grasp EGFR-TKI treatment, particularly after cytotoxic chemotherapy failure.

The use of next-generation sequencing (NGS) may overcome these limitations and provide accurate genotype information to improve treatment decisions [17, 18]. With technological advances, the NGS has facilitated multigene profiling with only nanogram-sized samples of DNA and has better sensitivity than traditional sequencing platforms [13, 18]. Approximately 70% of patients with lung cancer are diagnosed solely by examination of small biopsies and/or cytologic samples due to their advanced disease [19, 20]. Unfortunately, cytologic samples are inadequate for mutational analysis because of paucity of neoplastic cells and/or large cellular heterogeneity [21]. Buttitta et al verified the feasibility of EGFR mutation analysis on bronchoalveolar lavage and pleural fluid samples by NGS and found that it was much more sensitive than Sanger sequencing [22]. Since both of our patient's abdominal operations were performed in another hospital, we could not obtain samples of her intestinal metastasis. In addition, due to the testing costs, the samples were not subjected to repeat EGFR mutation testing.

Recent studies have revealed that intra-tumor heterogeneity affects key cancer pathways, driving phenotypic variation [23]. Genetic instability within the tumor cell population leads to the accumulation of additional mutations within single cells, eventually causing genetic diversity [24]. Thus, a number of genetically divergent clonal subpopulations exist, the most aggressive of which drive the tumor progression. The biopsy material we analyzed was only part of the tumor and could not completely represent the entire tumor's biological characteristics. This might be a possible reason why the second patient, who did not have a detected EGFR mutation, benefited from EGFR-TKI. Intratumor heterogeneity has significant implications for biomarker choice and poses a significant challenge to personalizing cancer medicine.

Genomic information reported as haplotypes rather than genotypes will be increasingly important for personalized medicine [25]. The two alleles in a single cell vary in single nucleotides or larger genomic ranges by substitution, insertion, deletion, or changes in copy number. Using the present mutation analysis method, allele information from each pair is comingled into one sequence of information. However, both allelotypes and allelotype combinations affect phenotypes as well as treatment effect. Therefore, studies of the correlation between haplotypes and phenotypes will be helpful for further EGFR investigations. EGFR haplotyping can generate more all-round and effective information than could the present mutation analysis [26].

In summary, our results and those in the literature suggest that metastases to the GI tract from lung cancer may not be as rare as previously thought. Thus, GI metastasis should be considered, especially in patients with abdominal symptoms. Through these two cases, we observed different clinical effects and prognosis after EGFR-TKI–based therapy, although the mechanisms remain to be further elucidated.

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Disclosure of conflict of interest

None.

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