Drifting *EGFR* mutation

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Introduction

It is generally accepted that somatic mutation in the *EGFR* gene can serve as a biomarker for predicting the efficacy of treatment with epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) in patients with non-small cell lung cancer (NSCLC). Recently, in fact, randomized phase 3 clinical trials have clearly proven that the EGFR-TKIs erlotinib and gefitinib are effective for first-line treatment of advanced NSCLC harboring *EGFR* gene mutation (1-3). *EGFR* mutation has been shown to have statistically significant correlation with progression-free survival, but not with overall survival, probably because of crossover to alternative treatments (4). However, the situation is not that simple. The efficacy of EGFR-TKI has been less evident when used as a second-line treatment for NSCLC patients who have been previously treated with platinum-based combination chemotherapy (5). The INTEREST phase 3 trial compared gefitinib and docetaxel in previously treated NSCLC patients (6). Although the hazard ratio for survival was lower in patients with tumors carrying *EGFR* mutation than in those with wild-type EGFR, no statistically significant interaction was reportedly demonstrated (7). A recent and important study conducted in China by Bai and colleagues has attempted to address this issue (8).

Disappearance of *EGFR* mutation after chemotherapy

Bai *et al.* (8) investigated the possibility that *EGFR* mutation status might change after chemotherapy. They analyzed the presence of *EGFR* gene mutation in paired plasma samples from 264 patients with advanced (stages IIIb and IV) NSCLC before and after two cycles of platinum-based first-line chemotherapy. *EGFR* gene mutations were detected in 34.5% of samples (91 of 264) obtained before the treatment, but in only 23.1% of samples (61 of 264) obtained after the treatment. The difference was statistically significant (P<0.001). Importantly, the *EGFR* mutation status in 20.5% of the patients (54 of 264) was found to be switched from positive (mutant) to negative (wild-type) after chemotherapy. Furthermore, these patients had a better response rate than patients showing a reverse change of mutation status (P=0.037). More recently, Honda *et al.* (9) reported the similar disappearance of an activated *EGFR* mutation in a Japanese woman after extensive treatment with EGFR TKIs and cytotoxic drugs. Although the patient's malignant pleural effusion and supraclavicular lymph node metastasis carried the same *EGFR* mutation (L747–T751 deletion in exon 19), no *EGFR* gene mutation was detected in pleural effusion obtained after the treatments. A pair of cell lines established from the effusion were confirmed to lack the *EGFR* gene mutation, and the pathological features of xenografts established from the cell lines resembled those of the original lymph node metastasis, indicating they were the same tumor. In the INTEREST trial mentioned above, the *EGFR* mutation status was determined from archived primary tumor samples (6). Therefore, there would have been a gap between the time points at which mutation status was determined and study entry. If we assume that first-line chemotherapy had changed the *EGFR* gene status, failure to detect any correlation between the original *EGFR* status and the efficacy of EGFR-TKI therapy would not be surprising. Therefore, the study reported by Bai *et al.* (8) has had a huge impact on oncological practice, and suggested that data obtained in clinical trials should be interpreted carefully.

Detection of *EGFR* mutation in plasma samples

It is often difficult to obtain biopsy samples sufficient for DNA sequencing from patients with unresectable NSCLC. However, currently available highly sensitive state-of-the-art methods have made it feasible to determine tumor genotypes using peripheral blood samples, thus providing a relatively non-invasive and repeatable source from which to obtain information on *EGFR* mutation status. However, discrepancy of *EGFR* mutation status between peripheral blood samples and matched tumor tissues has been reported. For example, Goto *et al.* (10) investigated *EGFR* gene mutation status based on circulating free DNA in serum versus tumor biopsy samples in Japanese patients who participated in the IPASS (IRESSA Pan-Asia Study) study. There was a significant association between *EGFR* gene status based on circulating free DNA and progression-free survival after first-line gefitinib treatment, but a high rate of false negativity (56.9%) was evident when the circulating free DNA was analyzed. In a previous report, Bai *et al.* (11) showed that the concordance of *EGFR* mutation status between 230 plasma samples and matched tumor tissue samples was 78%. This discrepancy could have accidentally led to underestimation of *EGFR* gene mutations in plasma samples obtained after chemotherapy. To eliminate this possibility, they carefully applied two methods: DHPLC (denaturing high-performance liquid chromatography) and ARMS (amplification refractory mutation system), but further confirmatory analysis will be needed.

EGFR mutation shift in tissue samples

Bai *et al.* compared the *EGFR* mutation status of paired initial diagnostic biopsy and surgically resected tumor samples from 63 NSCL patients who received two to four cycles of cisplatin-based neoadjuvant therapy (8). Consistent with the aforementioned analysis of plasma samples, *EGFR* mutations were detected in 34.9% (22 of 63) of patients before the neoadjuvant chemotherapy, but in only 19.0% (12 of 63) after the treatment. In 12 patients (19.0%) the EGFR status changed from mutant to wild type. Although the number of patients was smaller than that used for the plasma DNA analysis, these results clearly substantiated the shift of *EGFR* status after treatments with cytotoxic drugs.

Origin of cells with the wild-type *EGFR* gene in *EGFR* mutant tumors

The next question is the origin of different mutations from the same tumors. Although the growth of cancer cells has been considered clonal, there is now accumulating evidence for the presence of minor clones with different genetic profiles within the same tumor. To reveal intra-tumor heterogeneity, Bai *et al.* analyzed *EGFR* mutation status in more than 2,506 tumor foci microdissected from 79 tumors in patients with NSCLC who had undergone palliative surgery. Surprisingly, aproximately 38% of the tumors contained both *EGFR*-mutant and wild-type foci (8). Taniguchi *et al.* had earlier reported similar data (12). Although Yatabe *et al.* reported that heterogeneous distribution of *EGFR* mutations was extremely rare (13), the number of foci per person or the number of patients they examined were too small for statistical substantiation of their findings.

Emergence of drug-resistant tumor cells after treatment

It is often found that tumors initially showing a favorable response to drug treatment become refractory to the same drugs after continuation of the treatment. Intra-tumor genetic heterogeneity has been shown to be one of the major molecular mechanisms responsible for such acquired resistance of NCLC to EGFR-TKIs.

Emergence of the EGFR-TKI-resistant *EGFR* gene mutation T790M is one of the main causes leading to failure of EGFR-TKI treatments (14). A recent study using a highly sensitive method based on matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has revealed that minor populations of cells with T790M mutant alleles were already present in untreated tumors. Su *et al.* reported that T790M was detected in 25.2% (27 of 107) of untreated NSCLCs (15). T790M mutant cells are resistant to EGFR-TKIs, and are thought to become the prominent populations after EGFR-TKI treatment has eradicated the sensitive cells. Similar emergence of EGFR-TKI-resistant tumor cells with *MET* gene amplification has also been reported (16). We previously established a gefitinib-resistant subline from PC9 NSCLC cells bearing *EGFR* mutation (17). The gefitinib-resistant PC9 cells demonstrated increased Akt phosphorylation (not inhibitable by gefitinib), reduced expression of PTEN protein, and loss of the *EGFR* mutation. This result suggests that minor clones with different *EGFR* gene status may exist even in an established cancer cell line.

Conclusions

The *EGFR* gene status of a tumor changes as a result of chemotherapy. This concept is quite new and needs to be

validated independently by other investigators. Cytotoxic drugs used for chemotherapy are highly mutagenic and may cause new mutations, but are unlikely to repair the mutated *EGFR* gene. The presence of genetic heterogeneity within an untreated NSCLC is now accepted. The shift of *EGFR* mutation status after chemotherapy is likely to result from the emergence of minor cell populations differing in *EGFR* gene status. However, this hypothesis requires caution because it is based on the assumption that the sensitivity of tumor cells with *EGFR* mutation to cytotoxic drugs is higher than that of cells bearing the wild-type *EGFR* gene. Further studies will be necessary to fully elucidate the molecular mechanisms behind this genetic shift.

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