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Invited critical review

EGFR T790M resistance mutation in non small-cell lung carcinoma

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ABSTRACT

Lung cancer patients carrying sensitive epidermal growth factor receptor (EGFR) mutations show dramatic responses to tyrosine kinase inhibitors (TKIs). However, the majority of patients whose disease responds to drugs eventually develop resistance to these EGFR-TKIs. The T790M gatekeeper mutation in the EGFR tyrosine kinase domain accounts for half of resistance to these drugs. In some patients, this mutation is also detected as a primary event before drug exposure, at a frequency that is highly dependent on the technique used. This review will focus on the methods that have been used to detect the T790M mutation, and its potential clinical applications both in TKI naïve patients and in patients with an acquired resistance.

Contents

1. EGFR TKI and activating mutations

Prospective phase III randomized clinical trials showed that tyrosine kinase inhibitors (TKI) gefitinib [1–4], erlotinib [5,6], and more recently afatinib [7,8] improved outcomes compared with chemotherapy as initial treatment in EGFR-mutant non small cell lung cancer (NSCLC). These molecules have thus been approved in many countries worldwide. Therefore, routine molecular analysis of pathological specimens is mandatory in clinical practice to predict patient response. The potential result is an increased likelihood that patients will receive optimal therapy for their tumor and be spared a course of therapy with no or significantly less benefit.

Activating EGFR mutations occur in 10–15% of NSCLC patients of Caucasian ethnicity. They are more frequent in never-smokers, females, and adenocarcinomas [9]. EGFR mutations affect the EGFR tyrosine kinase domain, within exons 18–21. The p.L858R substitution in exon 21 and in-frame deletions in exon 19 account for 90% of all EGFR mutations. These are clearly activating mutations. Apart from these two hotspot mutations, other mutations have been described [10]. Substitutions in exon 18 (codon 719) are associated with TKI responses [11–13], although lower than those observed for exon 19 deletions and p.L858R mutation. Exon 19 insertions (in-frame insertions of 6 amino acids in the kinase domain) have been described as EGFR-TKI-sensitizing mutations as well [14]. The p.S768I mutation on exon 20 has initially been associated with low sensitivity to gefitinib and erlotinib in vitro [15], but good clinical responses have been reported in patients presenting an EGFR S768I alone [16] or associated with another EGFR alteration [17].

In current clinical practice, there is no standardized method for the detection of EGFR mutations in NSCLC tumor samples. The samples

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available for detection of somatic mutations in tumors are usually composed of mutant and wild-type DNA from tumor cells and wild-type DNA from non-malignant cells (normal epithelial cells, hematopoietic cells and stromal cells). Therefore there is a need for a sensitive technique and a complete reliable process. Standard dideoxy sequencing has been the "gold standard" for detecting somatic mutations in tissue samples. This method is robust but time-consuming, and it has only moderate sensitivity. Moreover it might suffer from a lack of robustness for the determination of mutations in formalin fixed paraffin embedded tumors [18]. These limitations of direct sequencing for detecting somatic mutations have led to the development of more sensitive, less expensive, and faster methods. A number of alternative procedures have therefore been developed to detect common cancer mutations, such as HRM [19–21], allele-specific amplification [9,22–24], primer extension [25], and pyrosequencing [26]. In most cases, a better sensitivity was obtained using targeted techniques as compared to direct sequencing [27,28] (for a recent review see Ellison et al. [29]).

2. EGFR alterations and primary resistance to EGFR TKI

Beside the activating mutations described above, some alterations have been associated with primary resistance to EGFR TKI. They are located on exon 20 of the EGFR gene, which encodes part of the kinase domain.

2.1. Exon 20 insertions

Exon 20 insertions account for 4–9% of all EGFR mutant lung tumors [30,31]. Most of these insertions occur between amino acids 767 to 774, within the loop that follows the C-helix of the kinase domain [32]. These alterations have been shown to confer resistance to EGFR TKIs [32–34].

2.2. T790M in untreated EGFR-mutant lung cancers

The T790M mutation results in an amino acid substitution at position 790 in EGFR, from a threonine to a methionine (Fig. 1). This mutation occurs within exon 20. Threonine 790 is the gatekeeper residue in EGFR. Its key location at the entrance to a hydrophobic pocket in the back of the ATP binding cleft makes it an important determinant of inhibitor specificity in protein kinases. Substitution of this residue in EGFR with a methionine has been thought to cause resistance by steric interference with binding of TKIs [35]. But the T790M mutation has also been shown to increase the ATP affinity of the oncogenic receptor mutant [36].

Germline T790M mutations have been observed at a low frequency (~0.5% of never smokers with lung cancer), sometimes in a context of familial cancer syndrome [37,38]. A prospective study of these patients and their families is under development, in order to define screening and counseling strategies for this rare but potentially high-risk population [39].

Somatic pre-treatment T790M mutations are in most cases associated in cis (i.e. on the same allele) to a sensitizing somatic EGFR alteration such as a L858R substitution or a deletion in exon 19. The most recent studies describing the frequency of baseline T790M mutation in EGFR-mutant tumors are reported in Table 1. The rate of pre-treatment T790M mutation appears to be highly dependent on the sensitivity of the method used. T790M is rarely found in untreated EGFR mutant tumors using conventional testing technique. Using Sanger sequencing, a method of relatively low sensitivity, studies report an incidence of baseline EGFR T790M of 0–5.9% in tumors presenting a sensitizing EGFR mutation [33,40–42]. When molecular methods with a higher sensitivity are utilized, the reported incidence of baseline EGFR T790M is greater. These sensitive methods include mass spectrometry-based mutation assays (2% [43]; 25.2% [42]), single allele base extension reaction (7% [44]), mutant-enriched PCR-based methods including the mutation-biased PCR quenching probe method (9.4% [45]), the Scorpion

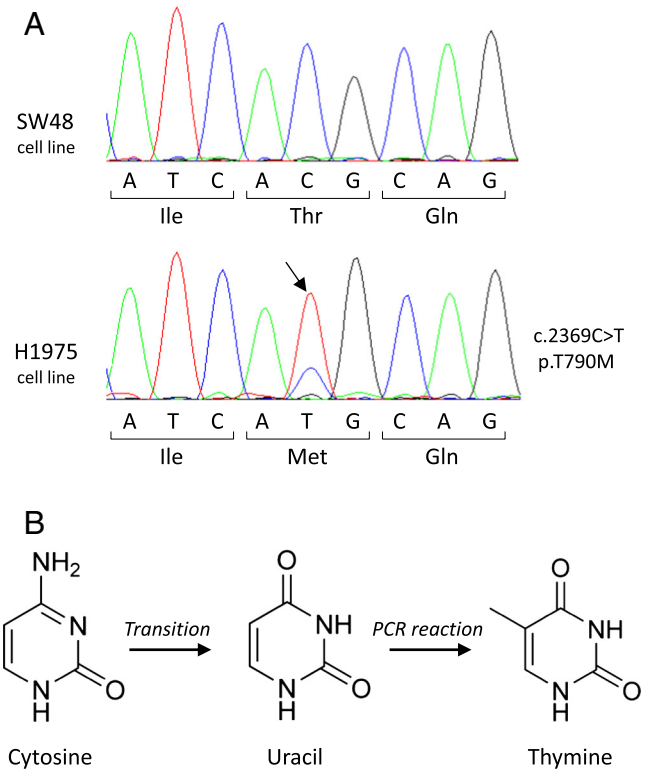

Fig. 1. (A) Detection of the T790M mutation by DNA sequencing. Partial sequences of EGFR exon 20 are presented. The mutated nucleotide is indicated with an arrow. (B) In FFPE tissues, cytosine can be transformed into uracil (transition), which in turn is converted to thymine during DNA synthesis.

Amplification Refractory Mutation System (SARMS) technology (38% [40]), and Taqman probes in the presence of a peptide-nucleic acid (34.9% [46]; 65.3% [47]). The highest prevalence (78.9%) was obtained using colony hybridization [48]. This highly sensitive method detects EGFR T790M at concentrations as low as 0.01% of the total DNA present.

These highly sensitive assays can detect minor EGFR T790M-containing clones. But there is also a potential for false-positive results. Using mass spectrometry-based assays, low DNA content and quality can lead to the generation of mutant peaks that can be indistinguishable from a true mutation [43]. With mutant-enriched PCR assays, the amplification of Taq errors can occur in very low template DNA samples leading to false-positive mutation calls as well. In addition, the use of formalin-fixed specimens for molecular testing may result in artificial mutation. Indeed, these fixation conditions have already been shown to induce transitions, i.e. point mutations that change a purine nucleotide to another purine (A > G) or a pyrimidine nucleotide to another pyrimidine (C > T), which is the case for the T790M mutation (Fig. 1). Such artifacts have been observed when performing PCR amplifications of small amounts of DNA, particularly if the DNA is isolated from formalin-fixed paraffin-embedded (FFPE) tissues [49–51].

In the case of the EGFR T790M mutation, this issue has been carefully addressed by Ye et al. [52]. Thirty-six TKI-naïve tumors harboring known sensitizing EGFR mutations with both frozen and FFPE samples were analyzed for EGFR T790M using a mutant-enriched PCR assay (0.1% analytical sensitivity). In the FFPE samples, the EGFR T790M-positive rate was 42% (15/36) in the tumor tissue. But a similar mutation rate (49%; 16/33) was found in the adjacent normal tissue. When using frozen samples, only 1 of 36 samples (3%) was EGFR T790M positive, and none of the 35 adjacent normal tissue samples were found to harbor T790M. The authors conclude that detection of T790M mutations may in some cases be FFPE-derived artifacts [52]. One way to limit them would be to treat the template with uracil-N-glycosylase, an enzyme that removes uracil from DNA [53].