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Original article

EGFR and *KRAS* molecular genotyping for pulmonary carcinomas: Feasibility of a simple and rapid technique implementable in any department of pathology

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ABSTRACT

Objectives: *EGFR* and *KRAS* genes are routinely tested in lung carcinomas with therapeutic implications. However the current testing methods require complex infrastructures and the delay for diagnosis remains often rather long, especially for initiating an appropriate treatment in patients with advanced stage tumor and short life expectancy.
Material and methods: We evaluated the Idylla™ fully automated molecular diagnostic system in routine conditions in 79 lung adenocarcinomas and 14 other non-small cell lung carcinomas, mostly in advanced stages (III or IV: 85%). Tests were performed on formalin-fixed paraffin-embedded ($n = 83$) or fresh ($n = 10$) material, including cytological ($n = 24$) and small biopsy ($n = 20$) samples. In prospective cases ($n = 82$), the most likely mutated gene (*EGFR* in non or occasional smokers and *KRAS* in smokers) was tested first; the second gene being only tested in case of negativity.
Results: The system did not require complex training. Mutational status was obtained in few hours after making the histological diagnosis and on the day of the patient's sampling by analyzing fresh material. The sequential testing strategy avoided 15 *EGFR* and 15 *KRAS* tests that would have been negative. Compared with reference methods, global specificity and sensitivity were both 100% for *EGFR* mutations, and 89.1% and 91.7% for *KRAS* mutations, respectively.
Conclusions: We demonstrated that such easy-to-use systems can permit pathologists to integrate a reliable *EGFR*/*KRAS* status in their initial pathologic report, and could be useful complementary tools to the current molecular diagnostic methods, with regard to prompt therapeutic management of lung cancer patients.

1. Introduction

Lung carcinomas are aggressive and frequent tumors [1]. About 75% are inoperable hat presentation. Recently, there have been major advances in the understanding of the pathogenesis and management of these carcinomas, with the discovery of the biological and therapeutic importance of pharmacologically targetable acquired genetic alterations [2].

Interesting genes that are routinely tested in lung carcinomas are those encoding for *EGFR* (epidermal growth factor receptor) and *KRAS* (Kirsten rat sarcoma viral oncogene) [2–7]. Mutations of these two genes almost exclusively occur in non-small cell and non-squamous cell carcinomas [8,9], which represent about 60% of lung carcinomas. These mutations are usually seen in different clinico-pathological settings. *EGFR* mutations occur in about 15% of cases in a Caucasian population, and more frequently in Asian, female or non-smoker patients and in TTF1-positive, non-solid and

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non-mucinous adenocarcinomas [10,11]. *KRAS* mutations occur in about 30% of cases and especially in smoker patients.

The interest of testing *EGFR* mutations is their association with therapeutic efficiency of tyrosine kinase inhibitors [12]. There is presently no direct therapeutic implication for *KRAS* mutations. However the interest of testing *KRAS* mutations lays mainly in their high frequency and in that their presence virtually excludes targetable mutations [10,13]. Other genes routinely studied in lung carcinomas (*ALK*, *BRAF*, *PIK3CA*, *HER2*, *ROS1*...) show abnormalities in less than 5% of cases.

Because of its complexity, genotyping of tumors is generally outsourced by the departments of pathology to outside molecular pathology laboratories or performed in large biopathological centers [5]. Many centers and countries still have no facilities and make the classical testing an issue in terms of duration and cost. External handling can be the cause of delays and complex genetic testing itself is time-consuming [7]. The resulting delays can be an issue for the oncologist initiating either targeted therapy or usual chemotherapy [5,14]. In a retrospective study of 18,679 routine molecular profiling of non-small cell lung cancer (NSCLC) performed in France during a 1-year period (2012–2013) [5], the median interval between tissue specimen collection and the initiation of molecular analysis was 8 days (interquartile range 4–16) and the median interval from the initiation of molecular analysis to the final written report with *EGFR* mutation was 11 days (interquartile range 7–16). In this study, the rather long turnaround time motivated the local multidisciplinary tumor board to plan the treatment strategy without consideration of molecular profile results in 836 of 3707 patients (23%). In a Canadian study, only 27/126 (21%) patients with biomarker testing had results at their initial oncology consultation and 8/43 (19%) with *EGFR* or *ALK* positive results started chemotherapy before biomarker results became available [14]. Large-scale multiplexed assays afford the capability of testing many genes at once [15] but this technology still takes long to complete and analyze. Turnaround time in routine clinical practice was 2.8 weeks (range 1.0–8.9 weeks) in a series of 552 genotyped cases with multiplexed assays [16].

Fortunately, the technology is rapidly improving [17] and will probably change the current practice. We have evaluated a newly marketed simple technique that can be used by all pathologists and can substantially shorten delays. We used this technique in a series of pulmonary carcinomas to rapidly assess the *EGFR* and *KRAS* molecular status. We have studied its real value in term of feasibility, accuracy and potential benefits.

2. Material and methods

2.1. Rapid testing system

EGFR and/or *KRAS* mutation status were assessed in the department of pathology at Marie Lannelongue Hospital, with the Idylla™ (Biocartis, Mechelen, Belgium) system. The details of this fully automated real-time polymerase chain reaction based system have been previously described [18,19]. This system requires a disposable cartridge for each test. The Idylla™ *EGFR* Mutation Assay, is intended for detection of 52 mutations in the *EGFR* oncogene: exon 18 (G719A/S/C), exon 21 (L858R, L861Q), exon 20 (T790M, S768I) mutations, exon 19 deletions and exon 20 insertions. The Idylla™ *KRAS* Mutation Test is intended for detection of 21 mutations in codons 12, 13, 59, 61, 117 and 146 of *KRAS* oncogene.

According to the manufacturer instructions, the *EGFR* tests were performed on a 5 μm thick section of formalin-fixed, paraffin-embedded (FFPE) tissue. For *KRAS* Mutation Test, one or several 5 μm thick sections corresponding to a total surface of 50 mm^2 were used, possibly with macrodissection if tumor cellularity was under 25%. When a diagnosis was obtained on per-operative cytology or frozen section, we also used fresh material: 20 μl of fluid, or frozen sections as FFPE ones.

2.2. Patients and tumors

Main patients' clinical data and main pathological data are summarized in Table 1.

Clinical data were obtained from the patients' hospital file. We tested 11 recent retrospective cases, which were selected because of their various known *EGFR* and *KRAS* status and of their various clinical diagnostic procedures. Prospective analyses were performed as complement of the usual pathological diagnostic procedures in 82 patients, between July 1st 2016 and February 28th 2017. Patients were informed and signed a written consent.

Carcinomas were classified according to the 2015 WHO classification [1]. Immuno-histochemistry and mucin stain (alcian blue) were performed if necessary, according to the current diagnostic recommendations [4]. Antibodies against TTF1, p63, Keratin 7 were used with a Ventana BenchMark GX automated stainer. Small cell carcinomas and squamous cell carcinomas, with very low probability of *EGFR* or *KRAS* mutation, were excluded. Cases with absolute or relative (tumor cellularity below 10%) insufficient tissue or cytological material were also excluded.

This study had the approval of our local Ethics Committee.

2.3. Testing strategy in prospective cases

Eighty cases were reflex testing (decided by the pathologist) and 2 were recently archived cases tested at the request of the clinicians. Non-metastatic and resectable cases were included only if there was a sufficient probability of *EGFR* mutation (TTF1-positive non-solid and non-mucinous adenocarcinomas in patients with no smoking history).

A sequential strategy was used according to the patient's smoking habit. In current or former smoker patients (more than 5 pack-year history), *KRAS* test was performed first, and *EGFR* status was assessed only if no *KRAS* mutation was detected. Conversely, in non- or occasional smoker patients (5 pack-year or less), *KRAS* mutational status was only performed if no *EGFR* mutation could be detected first.

The results were included in the initial pathologic report and given to the clinicians. Clinicians were informed that the Idylla™ *EGFR* Mutation Assay was intended for Research Use Only at the time of the study and that samples were sent for usual molecular analyses.

2.4. Control by reference methods

Usual molecular analyses were performed with Vela Diagnostics CE-IVD Sentosa® SQ NSCLC Panel for Next-Generation Sequencing at Gustave Roussy Universty Hospital. Samples that were sent were paraffin blocks for large surgical specimens or blank slides and paraffin shavings in other cases, according to our established routine process. Discordant cases were genotyped with allele-specific primer extensions and Sequenom iPLEX mass-spectrometry at Paul Brousse Hospital. False results obtained with the Idylla™ system were reviewed and interpreted by Biocartis.

3. Results

The *EGFR*/*KRAS* mutational status analysis could start as soon as the histological or cytological diagnosis of pulmonary adenocarcinoma or NSCLC carcinoma was established. The whole molecular diagnostic procedure lasted less than 3 h if a mutation was found in the first test and less than 6 h if the 2 genes had to be tested. In