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Original article

Assessment of EGFR mutation status in Tunisian patients with pulmonary adenocarcinoma

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ABSTRACT

Background. – Despite recent advances, non-small cell lung cancer carries a grim prognosis. For appropriate treatment selection, the updated guidelines recommend broad molecular profiling for all patients with pulmonary adenocarcinoma. Precise histological subtyping and targeted epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) testing are mandatory.
Methods. – Herein, we assessed the EGFR mutation status of 26 formalin fixed-paraffin embedded (FFPE) samples of lung adenocarcinoma. Mutational analysis concerned exons 18–21 of EGFR by real-time polymerase chain reaction (Real time-PCR) using the Therascreen EGFR RGQ PCR mutation kit. ALK status was established on 22 among 26 patients using D5F3 antibody with a fully automated Ventana CDx technique.
Results. – Activating EGFR mutations were found in 3 men among 26 patients (11.5%). Positive ALK expression was found in 2 cases among 22 patients (9.09%).
Conclusion. – Frequency of EGFR mutations in pulmonary adenocarcinomas of our series is similar to that found in the European ones with some particularities. The mutations detected are uncommon. Whereas, we found a high frequency of positive ALK expression in our series compared to frequency reported in literature. Further studies with larger Tunisian series are required to obtain more conclusive results.

1. Introduction

The last decade, we have seen major advances in our understanding of the pathogenesis and management of non-small cell lung cancer (NSCLC), adenocarcinoma in particular. The discovery of acquired genetic alterations in the epidermal growth factor receptor (EGFR) gene involved in growth factor receptor signaling, has changed the way these cancers are diagnosed and treated [1–8]. EGFR mutations are associated with sensitivity to the EGFR tyrosine kinase inhibitor (TKI) [9–13]. Subsequently, another cancer driver was discovered, a gene rearrangement in chromosome 2, leading to the fusion gene between Echinoderm Microtubule associated protein Like 4 (EML4) and anaplastic lymphoma kinase (ALK) [14]. Tumors harboring this fusion protein demonstrate impressive response rates when treated with the ALK inhibitor a first-line therapy comparing with first-line standard chemotherapy [14]. It is increasingly evident that the precise histological and molecular typing of NSCLC determines prognosis and the treatment. However, these molecular predictive tests are not currently universally available. This is the case of developing countries where management of NSCLC is constrained by lack of economic and healthcare infrastructure [15]. The present study is the first Tunisian one that evaluated mutation status of EGFR and ALK genes.

2. Methods

A series of 26 formalin fixed and paraffin embedded (FFPE) archived tissues were obtained from Tunisian patients with pulmonary adenocarcinoma at Department of Pathology in Charles Nicolle Hospital of Tunisia. The samples corresponded to 17 biopsies and 9 lung resection specimens.

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The proportion of tumor cells was estimated semi-quantitatively and a representative area was marked on hematoxylin and eosin-stained slides.

2.1. DNA extraction

The tumor tissues corresponding to the selected areas were isolated by manual microdissection and scraped into microcentrifuge tubes. The tissues were deparaffinized with 1 ml xylene at 56 °C for 10 min, washed with 1 ml 100% ethanol for 5 min at room temperature, and then dried at 37 °C for 10 min. QIAamp DNA FFPE tissue kit (Qiagen), was used to extract the genomic DNA from FFPE samples. DNA concentration and quality (A260/280 and A260/230) was measured by Nanodrop-2000, and then stored at −20 °C for further testing.

2.2. EGFR mutation testing

Mutation analysis of EGFR using the Therascreen EGFR RGQ PCR kit (Qiagen) was performed on the Rotor-Gene Q (Qiagen). The assay was based on the amplification refractory mutation system (ARMS) and on Scorpion technology. PCR reactions were carried out in a total volume of 25 µl (19.5 µl reaction mix + 0.5 µl *Taq* DNA polymerase + 5 µl sample) and the following cycle conditions: 95 °C for 15 min, 40 cycles at 95 °C for 30 s and 60 °C for 1 min.

The kit allows the detection of a wide scope of mutations, including 19 deletions between 2235 and 2258 in exon 19, three insertions in exon 20, the point mutations G719A/S/C (exon 18), S768I, and T790M (exon 20) and L858R and L861Q (exon 21) of EGFR. This assay was performed according to the manufacturer's guidelines and comprises a two-step procedure. In the first step, the control assay was performed to assess the total DNA in a sample. In the second step, both the mutation and control assays were performed to determine the presence or absence of mutated DNA. The total sample DNA was assessed by amplifying a region of exon 2 from EGFR by PCR. Next, the DNA samples were tested for the presence or absence of EGFR mutations Real time-PCR using a Scorpion probe and primers specific for wild-type and mutant EGFR DNA. The differences between the mutation assay cycle threshold (C_T) values were determined. Samples were classified as mutation positive if they give a ΔC_T less than the cut-off ΔC_T value. For each run, we ensured that our control analysis was not contaminated. We verified the internal controls of the reaction namely the negative control (NTC) and The EGFR Positive Control (PC).

2.3. ALK expression analysis

ALK protein expression was performed using D5F3 antibody (Ventana, D5F3) as previously described [16]. Sample was classified as positive if strong granular cytoplasmic staining was present in any percentage of tumor cells and as negative if the tumor cells displayed no or only weak or moderate cytoplasmic staining.

3. Results

3.1. Clinical data

The population consisted of 3 females (12%) and 23 males (88%). The average age was 58 years. All cases were pure adenocarcinomas that were classified as follows: 7 solid, 6 lepidic, 4 acinar, 2 colloid, 1 papillary, and 3 invasive mucinous adenocarcinomas (Table 1). In three cases, the histological suptype was not available. Smoking status was provided for 15 patients. The TNM classification of tumors and stage grouping were available for 8 patients (Table 1).

3.2. Analysis of internal controls

In our set, the testing was successful in all patients with validated controls. Our negative controls (NTC) have not generated a C_T value in the green channel (FAM) below 40. The positive control (PC) showed a control assay C_T of 26.26–30.95 in the green channel (FAM) (Fig. 1). The NTC and PC showed an amplification of C_T in 31–37 intervals in the yellow (HEX) channel (Fig. 2).

3.3. EGFR and ALK analysis

The cases with detected mutations have shown a positive FAM amplification and the ΔC_T were at or below the cut-off value. Activating EGFR mutations were found in 3 men (11.5%). One patient had exon 20 insertion, the second had G719X (A/S/C) point mutations in exon 18, and the third had a deletion in exon 19 (Table 1).

Two cases (9.09%) showed a strong granular, homogenous and diffuse cytoplasmic staining (Fig. 3).

4. Discussion

We detected activating EGFR mutations in 3/26 Tunisian patients (11.5%) with pulmonary adenocarcinomas using the

Table 1
Clinicopathological data and EGFR/ALK status of the patients.

Patients	Gender	Age	Histological subtype	EGFR status	ALK status	Specimens	pTNM/stage grouping	Smoking data
1	M	52	Acinar	EGFRwt	ALKwt	Biopsy	–	Yes
2	M	52	Acinar	EGFRwt	ALKwt	Biopsy	–	–
3	M	51	Lepidic	EGFRwt	ALKwt	Surgical	pT3N0M0/II	Yes
4	M	64	Solid	EGFRwt	ALKwt	Biopsy	–	Yes
5	M	42	Acinar	EGFRwt	ALKwt	Biopsy	–	–
6	M	75	Solid	EGFRwt	ALKwt	Biopsy	–	Yes
7	M	–	Colloid	EGFRwt	ALKwt	Biopsy	–	–
8	F	52	Invasive mucinous adenocarcinoma	EGFRwt	ALKwt	Surgical	pT1bN0M0/I	No
9	M	42	Solid	EGFR+	ALKwt	Biopsy	–	Yes
10	M	–	Solid	EGFRwt	ALKwt	Biopsy	–	–
11	M	55	Solid	EGFRwt	ALKwt	Surgical	T3N1M0/III	Yes
12	M	51	Solid	EGFRwt	ALKwt	Biopsy	–	–
13	M	68	Lepidic	EGFR+	ALKwt	Surgical	pT3N0M0/II	Yes
14	M	65	Lepidic	EGFRwt	ALKwt	Surgical	pT3N0M0/II	Yes
15	M	78	Lepidic	EGFRwt	ALKwt	Biopsy	–	Yes
16	M	59	Solid	EGFRwt	ALKwt	Surgical	pT2N0M0/I	Yes
17	F	30	Papillary	EGFRwt	ALK+	Biopsy	–	No
18	M	63	Colloid	EGFRwt	ALKwt	Surgical	pT3N0M0/II	–
19	M	87	Invasive mucinous adenocarcinoma	EGFRwt	ALKwt	Biopsy	–	–
20	M	67	Acinar	EGFRwt	ALK+	Surgical	pT1N0M1/IV	Yes
21	M	68	–	EGFRwt	–	Surgical	–	–
22	M	–	Lepidic	EGFRwt	ALKwt	Biopsy	–	–
23	M	74	Lepidic	EGFR+	ALKwt	Biopsy	–	Yes
24	M	37	Invasive mucinous	EGFRwt	–	Biopsy	–	–
25	M	–	–	EGFRwt	–	Biopsy	–	–
26	F	46	–	EGFRwt	–	Biopsy	–	No

Notes: M, male; F, female; EGFRwt, wild type EGFR; EGFR+, activating EGFR mutations; ALKwt, wild type ALK; ALK+, ALK positive expression; pTNM, patholohical TNM; Yes, smoke; No, do not smoke; –, not available data.

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