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High prevalence of ALK+/ROS1+ cases in pulmonary adenocarcinoma of adoloscents and young adults☆

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ABSTRACT

Objectives: To investigate prevalence and age-distribution of *ALK*- or *ROS1*-translocated adenocarcinomas in patients ≤50 years of age.
Materials and methods: Paraffin sections of pulmonary adenocarcinoma were analyzed for *ALK* (637 cases) and *ROS1* (376 cases) translocations using FISH, and for *EGFR* mutations (789 cases) using mutant-specific Real-Time PCR.
Results: ALK or *ROS1* fusions were detected in 55 of 637 cases (8.6%). When patients were stratified for age, it was found that six of six cases (100%) of lung adenocarcinoma diagnosed in patients <30 years of age were translocated for *ALK* (4 cases) or *ROS1* (2 cases). With the increase of age, there was a gradual decrease in the percentage of positive cases. In fact, *ALK*-translocated or *ROS1*-translocated cases were 5 of 17 cases (29%) in the 31–40 years age-group, 6 of 46 cases (13%) in the 41–50 years age-group, and 38 of 568 cases (7.0%) in patients older than 50 years. The six patients <30 years of age (5F/1M), including two pediatric patients (≤18 years old), presented with stage IV disease, were never or light smoker, and had no family history of pulmonary tumours. Four of the six patients, were treated with crizotinib and had an objective response.
Conclusions: Our findings provide evidence that *ALK* or *ROS1* translocations are crucial events in tumourigenesis of pulmonary adenocarcinoma of very young patients, including pediatric patients

1. Introduction

Pulmonary adenocarcinoma is extremely rare in children and adolescents. In the National Cancer Data Base of USA, 211 cases of pediatric pulmonary malignant tumours were registered in the period 1998–2011 [1]; in that series only 20 cases (9.4%) were classified as adenocarcinoma. In adults, a consistent fraction of pulmonary adenocarcinomas are due to mutually exclusive mutations/translocations which are supposed to play a driver role in malignant transformation [2]. *ALK or ROS1* translocations are present in 4–8% of pulmonary adenocarcinoma at any age [3–9], in 16% of cases occurring in patients ≤50 years of age [8], and in 47% of Chinese patients ≤30 years of age [10]. The observation that patients with *ALK* or *ROS1* translocated tumours are generally younger is well established, nevertheless no information is available for the few cases of pediatric pulmonary adenocarcinoma described in the literature.

In the present study we have investigated *ALK* and *ROS1* fusions in 69 cases of pulmonary adenocarcinoma occurring in Caucasian patients ≤50 years of age, including 2 pediatric cases. Our findings provide strong evidence that prevalence of *ALK*- or *ROS1*-translocated tumours had a peak (six of six cases) in patients less than 30 years old, and then gradually decreases reaching plateau values (7%) in patients >50 years of age.

2. Materials and methods

This is a single centre, retrospective study, performed according to the informed consent law of Italy. All patients were Caucasians, living in Italy, and were assisted at Sant'Andrea Hospital of Rome, a referral centre for lung tumours. Formalin-fixed/paraffin-embedded samples of pulmonary adenocarcinomas were classified according to 2015 WHO classification [11]. Paraffin sections of tumours were tested for *EGFR* mutations (n = 789 cases; median age = 68.0 ± 11.7; M:F = 1.39), *ALK* fusions (n = 637 cases; median

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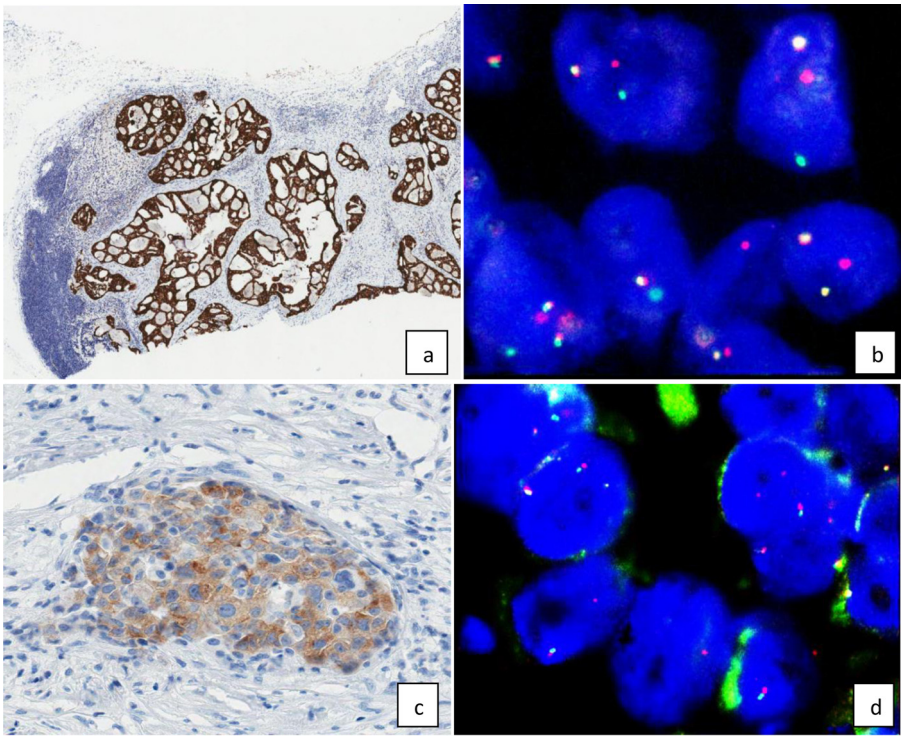

Fig. 1. Pediatric patient # 1. 17-years-old/M. Metastatic pulmonary adenocarcinoma (lymph node) with cribriform pattern. (a) Immunohistochemistry for ALK protein (D5F3; 3+). (b) FISH revealed 53% of ALK-translocated cells, 40% of which were also ALK-amplified. Tumor cells were TTF1+. The patient was treated with crizotinib, had a partial response, but died 9 months after diagnosis. Pediatric patient #2. 18-years-old/F. *trans*-thoracic needle biopsy with TTF1+ pulmonary adenocarcinoma solid-type. (c) Immunoohistochemistry for ROS1 protein (D4D6; 2+) revealed diffuse cytoplasmic staining (++) in tumour cells. (d) FISH analisys revealed ROS1 translocation in >15% tumour cells 25% of which were also ROS1-amplified. EGFR and KRAS were WT. Treatment with crizotinib induced a significant reduction of the tumour nodule (85%) and disappearance of brain metastasis; the patient is still treated with crizotinib and is in partial response 18 months after diagnosis.

age = 67 ± 12; M:F = 1.47), and *ROS1* fusions (376 cases; median age = 67 ± 13; M:F = 1.36) in the period 2012–2015.

EGFR mutations were tested using mutant-specific Real-Time PCR (*EGFR* Mutation Analysis Kit For Real-Time PCR, EGFR-RT52, ENTROGEN, CA, USA) able to identify 33 somatic mutations of exons 18, 19, 20, 21 of the EGFR gene. DNA was extracted from tissue sections. About 10 ng/ml of genomic DNA for each case was amplified. The mutations detected by the kit are: T790M, Exon 19 Deletions, L858R, L861Q, S768I, G719X, Exon 20 Insertions and Exon 21 Insertion.

ALK and *ROS1* fusions were determined by fluorescence in situ hybridization (FISH) on paraffin-embedded tissue sections according to protocols of Tissue Digestion Kit (KBI-60007, Kreatech, Resnova, Italy), using a break-apart probe specific to the *ALK* locus and to the *ROS1* locus (Vysis LSI ALK Dual Color, break-apart rearrangement probe and 6q22 ROS1 break-apart RUO kit; Abbott Molecular, Abbott Park). Positive cases were defined as those with >15% positive tumour cells.

Immunohistochemistry (IHC) for ALK protein (D5F3) and ROS-1 protein (D4D6) (Cell Signaling, USA) was used as a second, confirmatory technique in FISH-positive cases. Intensity of IHC-positive cells was graded as previously proposed [12]. All FISH-positive cases were also IHC-positive with intense/diffuse cytoplasmic staining (2+/3+).

2.1. Statistical analysis

X^2/Fisher's exact test was used to assess the association between gene alterations and age of the patients. t-student test was used to compare the mean age of patients with or without gene alterations.

3. Results

3.1. Age-distribution of ALK+ or ROS1+ tumours

ALK and *ROS1* translocations were investigated in a total of 637 cases of EGFR-WT primary pulmonary adenocarcinoma using FISH. The mean age of 47 patients (7.4%) with *ALK*+ tumours (55.7 ± 15.0 years) was significantly lower ($p < 0.001$; *t* test) than that of 590 patients with *ALK*-negative tumours (66.6 ± 11.33 years). Similarly, the mean age of 8 patients (2.1%) with *ROS1*+ tumours (46.6 ± 21 years) was significantly lower ($p < 0.001$; *t* test) than that of 368 patients with *ROS1*-negative tumours (65.2 ± 12.1 years).

When patients were stratified for age it was found that 78 patients (9.9%) were younger than 50 years (Table 1). Surprisingly, six of six patients less than 30 years old (5F: 1M) had tumours translocated for *ALK* (4 cases) or *ROS1* (2 cases); two of them were still in pediatric age (≤18 years). Moreover, it was noted that increasing age, there was a gradual and progressive decrease in the percentage of positive cases. In fact, *ALK*+ or *ROS1*+ cases were 5 of 17 cases (29%) in the 31–40 years age-group and 6 of 46 cases (13%) in the 41–50 years age-group. Patients older than 50 years (n = 568) had 5% *ALK*+ tumours and 1% *ROS1*+ tumours.

The age distribution of *ALK*+ or *ROS1*+ tumours was compared to that of 126 cases of *EGFR*-mutated adenocarcinoma (Table 1). It was found that prevalence of *EGFR* + cases was poorly influenced by age. In fact, 126 *EGFR*-mutated cases had a mean age of 67.0 ± 11.3 which was similar to that of 663 *EGFR*-WT cases (68.0 ± 11.7). The youngest patient with an *EGFR*-mutated tumour in our series was a 35-years-old female.