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EGFR and KRAS mutation coexistence in lung adenocarcinomas

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Lung cancer is one of the most common causes of cancer deaths. The development of EGFR targeted therapies, including monoclonal antibodies and tyrosine kinase inhibitors have generated an interest in the molecular characterization of these tumors. KRAS mutations are associated with resistance to EGFR TKIs. EGFR and KRAS mutations have been considered as mutually exclusive.

This paper presents three bronchial-pulmonary carcinomas, two adenocarcinomas and one pleomorphic sarcomatoid carcinoma, harboring EGFR and KRAS mutations.

Case 1 corresponded to an adenocarcinoma with EGFR exon 21 mutation (L858R) and KRAS codon 12 point mutation (G12V); case 2, a mucinous adenocarcinoma expressed coexistence of EGFR exon 21 mutation (L858R) and KRAS codon 12 point mutation (G12V); and case 3 a sarcomatoid carcinoma with EGFR exon 19 deletion – del 9bp and KRAS codon 12 point mutation (G12C - cysteine).

Based on our experience and on the literature, we conclude that EGFR and KRAS mutations can indeed coexist in the same bronchial-pulmonary carcinoma, either in the same histological type or in different patterns. The biological implications of this coexistence are still poorly understood mainly because these cases are not frequent or currently searched. It is therefore necessary to study larger series of cases with the two mutations to better understand the biological, clinical and therapeutic implications.

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Introduction

Lung cancer is one of the most common causes of cancer deaths [1,2]. Despite improvements in diagnostic and surgical techniques and chemotherapy protocols, overall survival is still low [3,4]. The NSCLC designation includes different carcinoma types such as squamous cell carcinoma, adenocarcinoma, large cell carcinoma and pleomorphic carcinoma [5,6]. Lung adenocarcinomas account for about 28% and 42% of the bronchial-pulmonary carcinomas diagnosed in women and men respectively [6]. The newly proposed lung adenocarcinoma classification recognizes a lepidic pattern instead of bronchiole-alveolar pattern, acinar, solid, papillary, micropapillary patterns as well as mucinous adenocarcinomas. This classification reinforces the importance of pattern recognition in lung adenocarcinoma diagnosis [6].

The development of EGFR-targeted therapies, including monoclonal antibodies and tyrosine kinase inhibitors, has generated interest in the molecular characterization of these tumors. Patients with lung cancer who benefit from EGFR tyrosine kynase inhibitors (EGRF TKI) may show dramatic responses with gefitinib or erlotinib therapy [7-11].

Activating EGFR mutations have been identified to predict EGRF TKI response [12-16].

KRAS mutations have been associated with EGFR TKIs resistance [17,18].

EGFR and KRAS mutations have been considered as mutually exclusive, as the KRAS-MAPKinase signaling pathway is also one of the signaling pathways for EGFR [18-20].

The objectives of this paper are to present three cases of bronchial-pulmonary carcinoma with coexisting EGFR and KRAS mutations and to discuss the clinical-pathological and therapeutic implications.

Material and Methods:

Material

This paper presents two adenocarcinomas and one pleomorphic carcinoma, harboring EGFR and KRAS mutations. Adenocarcinoma patterns present were registered.

The tissue for the analysis was obtained from 4% formalin-fixed paraffin embedded sections of lung surgical specimens and transthoracic biopsy (third case). All patterns present were manually dissected for selection after identification on hematoxylin – eosin stained slides.

Methods

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EGFR exons 19 and 21 and KRAS codon 12 and 13 mutations were evaluated after DNA extraction and polymerase chain reaction (PCR) amplification; EGFR exon 19 was studied by fragment analysis and EGFR exon 21 and KRAS codons 12 and 13 were studied by Sanger direct sequencing. Tissue representative of the adenocarcinoma patterns was selected after identification on HE stained slides and manual dissection of the tissue. The percentage of neoplastic cells was registered (in the three cases it was over 50%).

Genomic DNA was extracted from 5 µm section of paraffin-embedded tissue. For that, the QIAmp DNA Mini Kit (Qiagen, IZAZA, Germany) was used. One hundred nanograms (ng) of DNA was amplified in a 50 µl reaction solution containing 5 µl of 10x buffer (Roche, Germany), 2.5 mM MgCl2, 0.2 µM of each complementary primer, 200 µM deoxynucleoside triphosphate and one unit of DNA polymerase (Roche, Germany). A 5-minute initial denaturation at 95ºC was used to perform the amplifications; this was followed by 40 cycles of 30 seconds at 95ºC, 1 minute at 60ºC (for exon 19) or 57ºC (for exon 21), 1 minute at 72ºC and 10 minutes of final extension at 72ºC. The EGFR gene mutations located at exons 19 and 21 were determined using the intron-based primers according to the published method [21]. EGFR mutations were analyzed / detected according to the published method [22]. Exon 19 deletion was determined by common fragment analysis using PCR with an FAM-labeled primer set, and the products were submitted to electrophoresis on ABI PRISM 3100 (Applied Biosystems®). All electropherograms were reanalyzed by visual inspection in order to check for mutations. To evaluate the L858R mutation, MyCcycler (Bio-Rad) was also used, and its products were then studied by direct sequencing. The same procedure was applied to KRAS except for amplification which was performed using 5-minute initial denaturation at 95ºC; followed by 40 cycles of 30 seconds at 95ºC, 1 minute at 53ºC, 1 minute at 72ºC and a 10 minutes of final extension at 72ºC.

The same DNA samples were used for EGFR and KRAS mutational testing. The results were confirmed by double checking after another PCR reaction. Rules to avoid contamination were applied. Positive and negative (WT) controls were used in every test. Before sequencing an electrophoresis in agarose gel was performed to check the blank. Since 2012 our laboratory has participated in the European Society of Pathology (ESP) Lung External Quality Assessment Scheme (EQA) to ensure optimal accuracy and proficiency in lung cancer biomarker testing. Our score has been consistently ≥90%, considered as a successful participation.

Results

Case 1

A 77-year-old man had a 3 cm central tumor in the left lower lobe, corresponding to an adenocarcinoma with acinar, lepidic, solid and mucinous patterns, pT1bN0.

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EGFR exon 21 mutation (L858R) was present in the acinar and lepidic patterns (Figure 1).

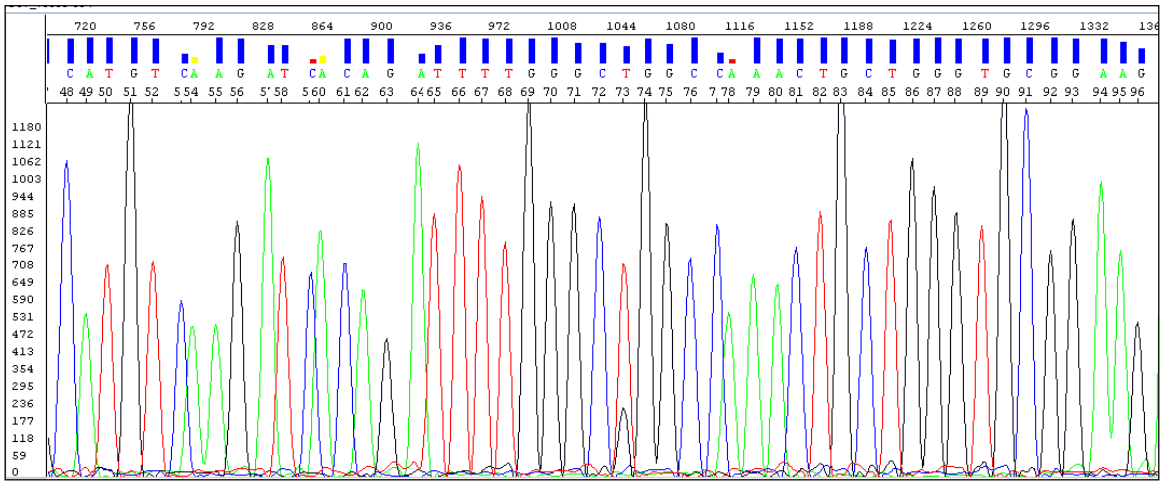

Figure 1. Exon 21 (L858R) EGFR mutation.

EGFR exon 19 was Wild Type (WT) in all patterns.

KRAS codon 12 point mutation (G12V) was also present in the acinar and lepidic patterns (Figure 2) (Table 1).

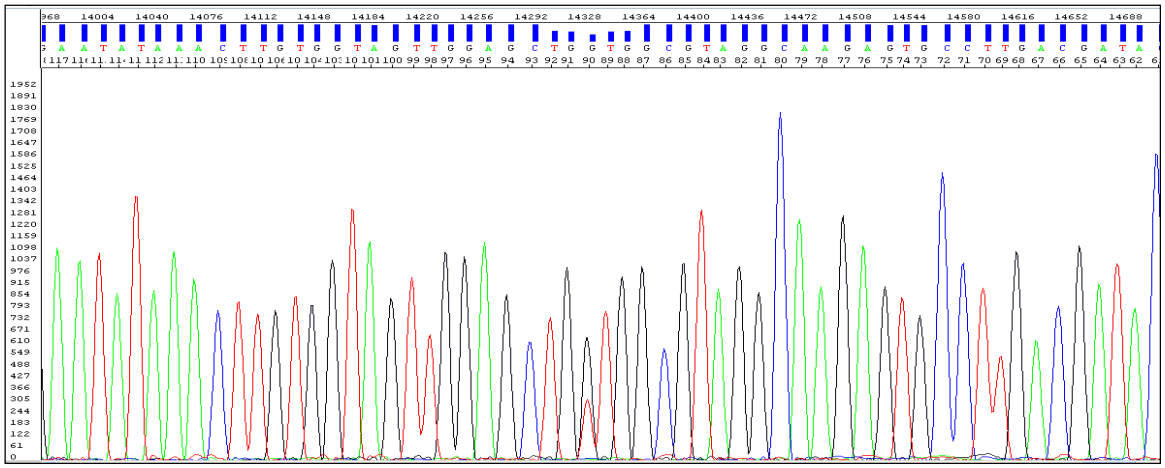

Figure 2. Codon 12 KRAS (G12V) mutation.

Case 2

A 77-year-old woman had a 6cm right upper lobe mucinous adenocarcinoma (with mucinous lepidic and mucinous acinar patterns), pT2b N0.

EGFR exon 21 mutation (L858R) was present in both patterns, while EGFR exon 19 was WT.

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KRAS codon 12 point mutation (G12V) was also concomitant (Table 1).

Case 3

A 76-year-old man had an 8 cm nodule in the right lower lobe with nodular pleural invasion as well as malignant pleural effusion, staged as pT3N0M1a. A transthoracic biopsy revealed a pleomorphic carcinoma, with acinar and giant/large cells.

EGFR exon 21 was WT and exon 19 deletion – del 9bp was present in both patterns (Figure 3) (Table 1).

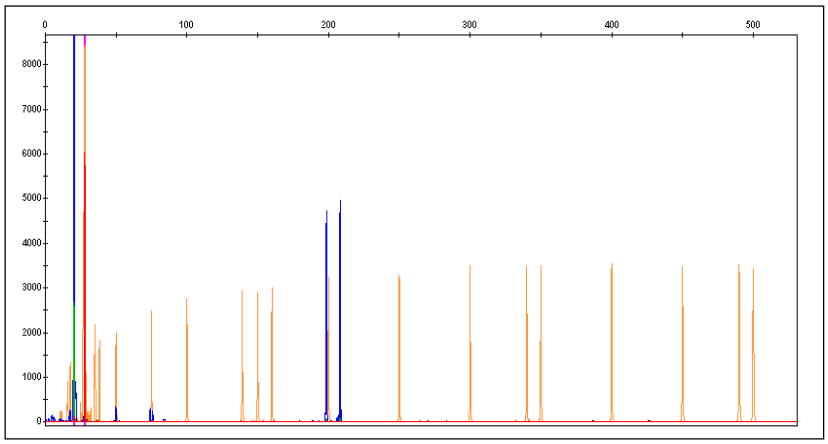

Figure 3. Exon 19 (Del 9bp) EGFR mutation.

KRAS codon 12 point mutation (G12C - cysteine) was also present in both patterns (Figure 4) (Table 1).

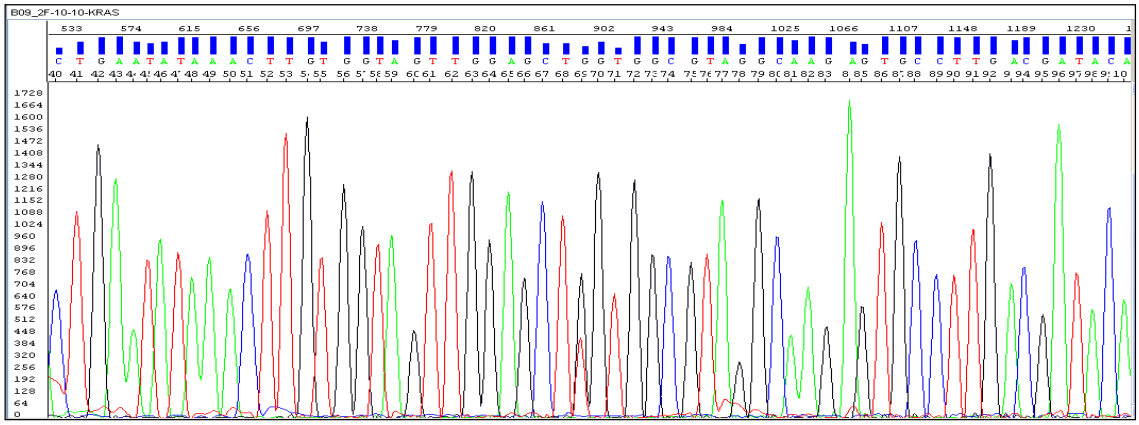

Figure 4. Codon 12 KRAS (G12C - cysteine) mutation.

Table 1: EGFR and KRAS mutations identified according to histologic patterns. WT – wild type; L858R - exon 21 EGFR point mutation; G12V – codon 12 KRAS mutation G12V; G12C – Codon 12 KRAS mutation G12C.

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Case 1	Mutations /patterns	Acinar	Lepidic mucinous	Solid	Mucinous
	Exon 21 EGFR	L858R	L858R	WT	WT
	Exon 19 EGFR	WT	WT	WT	WT
	KRAS	G12V	G12V	WT	WT
Case 2	Mutations /patterns	Acinar mucinous	Lepidic mucinous		
	Exon 21 EGFR	L858R	L858R		
	Exon 19 EGFR	WT	WT		
	KRAS	G12V	G12V		
Case 3	Mutations /patterns	Acinar	Giant cells		
	Exon 21 EGFR	WT	WT		
	Exon 19 EGFR	del 9bp	Del 9bp		
	KRAS	G12C	G12C		

Discussion

EGFR and KRAS mutations are considered as driver mutations because they are responsible for the initiation, progression and maintenance of lung cancers. Since 2004 the scientific community has known that pulmonary carcinomas harboring EGFR mutations are highly sensitive to EGFR TKIs [15].

Several studies have shown that the classical brochioloalveolar carcinomas and mixed-type adenocarcinomas have more frequently EGFR activating mutations [23-25]. Other studies have demonstrated that papillary, micropapillary and hobnail patterns are correlated with EGFR mutations [26, 27].

Dacic et al. demonstrated that EGFR mutated mixed-type adenocarcinomas showed the following predominance of primary histological patterns: acinar (20%), BA/lepidic (12.5%), mucinous (9%), papillary (3%), micropapillary (3%) and solid (0%); and KRAS mutated mixed-type adenocarcinomas showed acinar (32%), solid (18%), BA/lepidic (16%), mucinosous (16%), papillary (10%) and micropapillary (4%) [28]. Dacic et al. also demonstrated that tumors with a lymphocytic prominent response are less prone to have EGFR mutations and more likely to harbor KRAS mutations [28].

Intra - tumors heterogeneity for EGFR mutations has also been demonstrated [29,30]. KRAS mutations have been more frequently associated with mucinous differentiation, including mucinous BA/lepidic, acinar patterns, goblet cell morphology and poorly differentiated adenocarcinomas, especially in adenocarcinomas with solid patterns [28,31-37]. KRAS mutations are rare in squamous cell carcinoma and significantly more frequent in proximal lung adenocarcinomas (the bronchial type TTF1 negative) than EGFR mutations [38,39].

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Our two adenocarcinoma cases showed one lepidic pattern (second case) and the other (first case) mixed patterns where EGFR mutations are supposed to be more frequent. However the lepidic case had mucinous differentiation. KRAS mutation was present in the same area/patterns of the mixed adenocarcinoma (first case), namely in the acinar and lepidic patterns but not in the solid or mucinous patterns. In the second case it was present in the mucinous lepidic pattern, in accordance with the most frequent patterns harboring KRAS mutations in the literature.

In our laboratory, taking these three cases into account, we have a rate of 2.1% EGFR and KRAS coexistence. In our laboratory, the overall mutation rate for EGFR is 20% and for KRAS is 11%.

None of our patients underwent EGFR TKI therapy, so we cannot discuss the therapeutic consequences of EGFR and KRAS mutation coexistence in our cases.

EGFR mutations define a group of lung cancer which are dependent on EGFR signaling pathways and are more responsive to EGFR TKIs. EGFR mutations may also be a positive prognostic factor for advanced tumors treated with erlotinib [40].

Recent works have also demonstrated that EGFR mutational status is associated with sensitivity to first-line EGFR TKI in patients with advanced staged tumors [41].

Iressa Pan-Asia Study provided evidence that EGFR mutations are predictive of longer progression-free survival when treated with gefitinib compared with conventional chemotherapy protocols [42, 43].

The major signaling pathways for EGFR are RAS/MAPK and PI3K/AKT, with implications for cell proliferation, differentiation and survival [44]. Mutations on the downstream effectors of those pathways could be responsible to EGFR TKIs resistance [45,46]. KRAS is an important downstream effector in the MAPK pathway. Concomitancy of KRAS and EGFR mutations could seem redundant in their functional results. Mutant EGFR selectively activates AKT and STAT signaling pathways, which promotes cell survival [8].

KRAS mutations are associated with an unfavorable response to EGFR TKIs and resistance to conventional adjuvant chemotherapy with cisplatin/vinorelbine [18,47-50]. The TRIBUTE study also demonstrated that patients with KRAS mutation treated with erlotinib failed to benefit from erlotinib plus chemotherapy [40].

Evidence suggests that a tumor can harbor EGFR and KRAS mutations, which means that upstream inhibition of EGFR will have no therapeutic effect in these cases [51].

Recently Jackman et al. found no impact of KRAS mutations on the overall survival in patients without EGFR mutations treated with EGFR TKIs [41]. The role of KRAS mutation as a negative predictor of response of lung cancer treated with cetuximab is not clear either [52, 53]. Because EGFR mutation is a predictor of EGFR TKIs response and EGFR and KRAS mutations are generally mutually exclusive, it is not clear whether the response to TKIs differs between tumors with KRAS mutations and those without KRAS and EGFR mutations [54].

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There is a report of two cases of patients with colonic adenocarcinoma metastatic to lung EGFR WT and with KRAS mutation with durable responses to erlotinib [30]. There are other rare cases of TKI minor or transient response in patients with KRAS mutation [41,55]. Choughule et al described in their series three patients with KRAS and EGFR mutations with partial response to EGFR TKI [56]. Benesova et al also found response to gefitinib in three of five patients with this coexistence [57]. These facts raise the idea that KRAS mutation is not always associated with a lack of efficacy of EGFR TKI. The authors hypothesize that EGFR activation is not only mediated through KRAS signaling but also involves other pathways like PI3K/AKT/mTOR, phospholipase C or STAT. Negative feedback loops could also down regulate RAS signaling pathways [30]. Another possible explanation includes eventual concomitant molecular abnormalities that could activate EGFR [30]. Epigenetic variations, alternative splicing or posttranslational modifications could explain non active KRAS isoforms [30]. These authors therefore conclude that a small portion of patients with KRAS mutations might paradoxically benefit from EGFR TKIs.

One patient with KRAS mutations in the Southwest Oncology Group S0126 trial study responded to gefitinib, but none of four patients with both EGFR and KRAS mutations responded [58].

Zhu et al. also demonstrated that one patient with KRAS mutation responded to EGFR TKI [55]. The tumor had EGFR amplification but not mutation. It is argued that tumors with KRAS mutation are unlikely to respond to EGFR TKIs unless they have EGFR amplification [55].

Not all lung cancers with KRAS mutations are addicted to KRAS, demonstrated by in vitro studies [59]. Dependency was correlated with KRAS overexpression while the well-differentiated epithelial phenotype is correlated with RAS dependency [59].

Pao et al. first suggested that EGFR and KRAS mutations are mutually exclusive [18]. Other studies have also shown mutual exclusivity. Nevertheless combined EGFR and KRAS mutations do exist, though they are rare [40, 55, 56, 60, 61]. Li et al showed that when there was EGFR and KRAS mutation coexistence, EGFR mutations were more frequently identified in exon19 [61].

In our study, the EGFR and KRAS mutations present were always activating mutations, two identified in exon 21 and one in exon 19. These cases demonstrate that EGFR and KRAS mutations can coexist even in the same patterns of adenocarcinoma, although they have been discerned as mutually exclusive.

Lung adenocarcinoma comprises a group of tumors that are heterogeneous as regard histopathology typing, patterns and genetic alterations; hence, it is important to review a large number of histological sections to characterize the patterns present and even the whole tumor. In future, a more accurate molecular profile needs to be defined to overtake tumor morphological and genetic heterogeneity in order to explain the coexistence of KRAS and EGFR mutations.

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In the second case reported here, the EGFR mutation occurred in the lepidic pattern, although with mucinous differentiation. Nevertheless, there are EGFR mutations described in mucinous patterns. KRAS mutation is described as frequent in this pattern.

In the first case, the mutations were present in acinar and lepidic patterns and not in solid and mucinous patterns. Thus, tumor heterogeneity does not explain all the cases of EGFR and KRAS mutation coexistence. In these cases, genetic instability could explain the simultaneous or possibly sequential occurrence of the two mutations. Different clones of cells present in the same patterns could also explain the coexistence. Nor should we exclude the possibility of coexistence in the same cells that could overcome the downstream KRAS mutations by alternative signaling pathways or other regulatory mechanisms.

In the third case, EGFR and KRAS mutations were present in a pleomorphic sarcomatoid carcinoma. We know that KRAS mutations are more frequent in less differentiated lung cancers. These tumors are biologically aggressive and more prone to accumulate genetic instability and alterations.

Pleomorphic and sarcomatoid carcinomas are rare. One study revealed no EGFR mutations and a frequency of 38% KRAS mutations in primary sarcomatoid lung carcinomas. However, high EGFR polysomy (23%) and EGFR protein overexpression were identified in all the cases. These authors hypothesized that EGFR overexpression and KRAS mutation could explain aggressive biological behavior and worse prognosis of these subset of NSCLC [62].

Conclusions

Our findings strongly support the idea that EGFR and KRAS mutations can coexist in the same lung cancer, even in the same cell type of an adenocarcinoma. The biological implications of this coexistence are still poorly understood, mainly because these cases are not frequent, and bronchial-pulmonary carcinomas are not routinely studied in this extensive manner. In the future, studies of larger case series are required to better understand the biological, clinical and therapeutic implications of the discussed molecular alterations, based on uniformly designed studies.

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Authors' contributions

VS was responsible for drafting the manuscript, diagnosis, data collection and interpretation. LC was responsible for diagnosis, orientation and corrections. MS, AMA, TF and AL carried out technical issues and mutational status evaluation.

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