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About concomitant *KRAS* and other molecular alterations in non–small cell lung cancers

To the Editor,

We read with interest the study by Shaukat et al reporting about the correlations between molecular findings and tumor characteristics in the field of *RAS*-mutated non–small cell lung cancers (NSCLC). Among 220 tumors analyzed, they report 58 (26.36%) cases of *KRAS* mutations and 7 (3.18%) cases of *NRAS* mutations. In their series, *RAS* mutations were diagnosed only in *EGFR*, *BRAF* and *ALK* wild-type cases, which is concordant with the concept that driver oncogenic alterations are almost consistently mutually exclusive in NSCLC [1]. Nevertheless, double mutants have been reported, including cases with *KRAS* mutations [2]. In this letter, we aim to report the frequency of double mutants (ie, *KRAS* and another oncogenic alteration) in a case series of patients with advanced NSCLC tested for *EGFR*, *ALK*, *ROS1*, *BRAF* and *KRAS* alterations for therapeutic purposes.

The Brest Molecular Genetics Platform of Cancer located in the Brest University Hospital performs the molecular tests for NSCLC tumor samples of patients treated at the Brest University Hospital but also in other regional institutions from western Brittany. The panel of tests includes *EGFR*, *BRAF*, *KRAS*, *ALK* and *ROS1*. From January 2015 to October 2018, among 1909 NSCLC samples, *KRAS* mutations were identified in 660 (34.6%) samples including 546 (28.6%) mutations in codon 12, 56 (2.9%) mutations in codon 13, 46 (2.4%) mutations in codon 61 and 12 (0.6%) other mutations. In these *KRAS* mutants, another targetable oncogenic mutation was identified in 10 cases (ie, 1.5% of *KRAS*-mutated cases) with 6 *EGFR* mutations, 1 *BRAF*V600E mutation, 3 *ALK* rearrangements but no *ROS1* rearrangements. Whether the 2 driver mutations coexist in the same cells or are contained by different clones remains unsolved. Immunohistochemistry could help solve this question by localizing the activated/mutated oncogenic proteins at the single-cell level among tumor samples. Nevertheless, the variants of *KRAS* did not allow the study of the presence of the KRAS mutant protein at the cell level. Indeed, to the best of our knowledge, the only validated mutation-specific immunohistochemistry in the field of *RAS* mutations is the anti-RASQ61R clone SP174 test [3,4]. In our series, none of the double mutants had a *KRAS*Q61R mutation, and, consequently, we were not able to further study the expression of KRAS mutant proteins in double-mutated cases.

Among cases of NSCLC genotyped in our institution, we have encountered one case of *KRAS*Q61R-mutated NSCLC, reported to be very rare according to the COSMIC database (about 0.015% of NSCLC, https://cancer.sanger.ac.uk/cosmic). SP174 immunohistochemistry was performed on two biopsy samples from this case and revealed a homogeneous staining of every tumor area, suggesting the diffuse presence of the mutation in every tumor cell within the sample (Figure). Nevertheless, we cannot extrapolate this single result to every *RAS*-mutated NSCLC and notably to double mutants.

In conclusion, beyond the classical mutual exclusivity of driver molecular alterations in NSCLC, some samples can nevertheless contain double mutations. The necessity of diagnosing double mutations emphasizes the need to analyze concurrently the panel of *EGFR*, *BRAF*, *ALK*, *ROS1* and *KRAS* genes [5].

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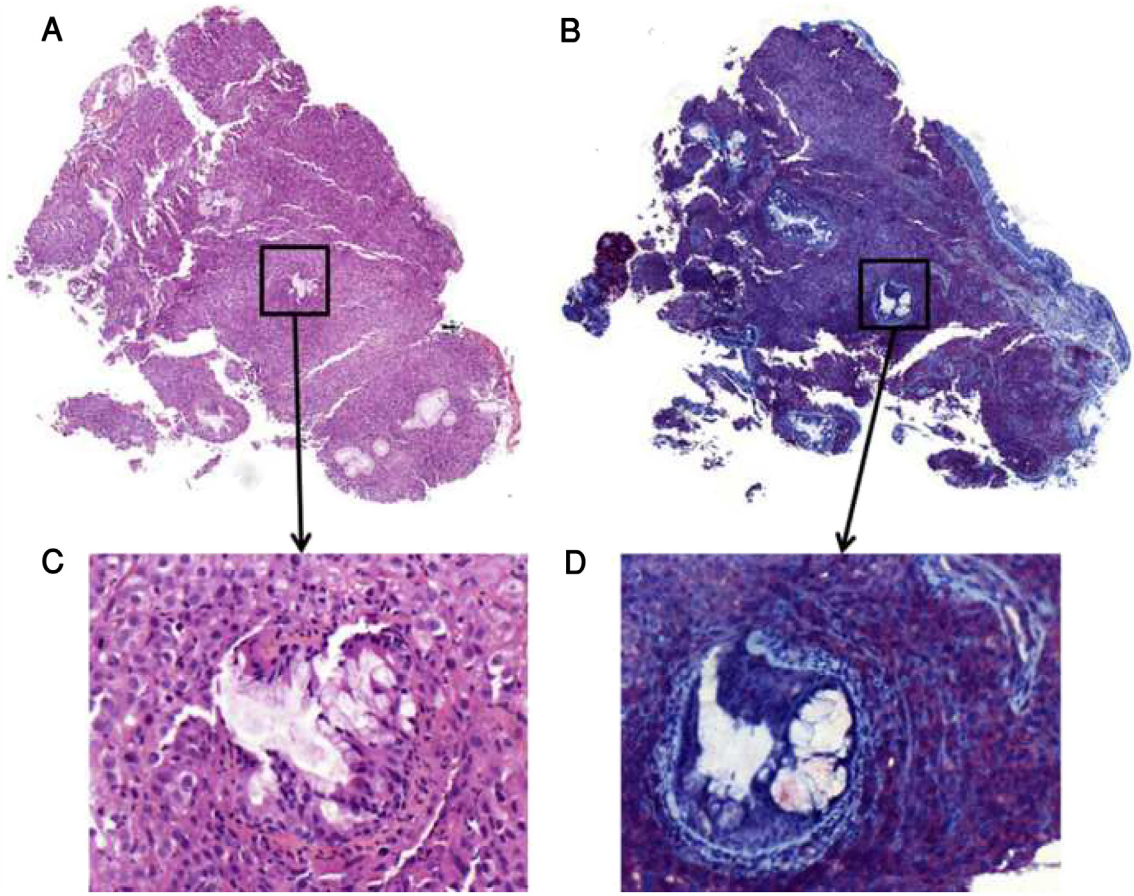

Figure Pathological analysis of a biopsy of an interesting *KRAS*Q61R-mutated non–small cell lung cancer. A and C, Poorly differentiated lung adenocarcinoma with entrapped non-tumor gland (hematoxylin-eosin-saffron; A, 30× magnification; C, 200×). B and D, The tumor cells present a diffuse red cytoplasmic staining using anti-RASQ61R immunohistochemistry around a non-tumor gland serving as an internal negative control (clone SP174, Red revelation, hematoxylin-counterstaining; B, 30×; D, 200×).

References

[1] Shaukat I, Kern JJ, Höti N, et al. Detection of RAS and RAS-associated alterations in primary lung adenocarcinomas. A correlation between molecular findings and tumor characteristics. Hum Pathol 2019;84:18-25.

[2] Van Der Steen N, Mentens Y, Ramael M, et al. Double trouble: a case series on concomitant genetic aberrations in NSCLC. Clin Lung Cancer 2018;19:35-41.

[3] Uguen A. About RAS mutation-specific immunohistochemistry in formalin-fixed, paraffin-embedded tumor samples. Hum Pathol 2017;60:208-9.

[4] Felisiak-Goląbek A, Inaguma S, Kowalik A, et al. SP174 antibody lacks specificity for NRAS Q61R and cross-reacts with HRAS and KRAS Q61R mutant proteins in malignant melanoma. Appl Immunohistochem Mol Morphol 2018;26:40-5.

[5] Ettinger DS, Aisner DL, Wood DE, et al. NCCN guidelines insights: non-small cell lung cancer, version 5.2018. J Natl Compr Canc Netw 2018;16:807-21.