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KRAS insertions in colorectal cancer: What do we know about unusual *KRAS* mutations?☆

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ABSTRACT

Introduction: *KRAS* mutations are negative predictors of the response to anti-EGFR therapy in colorectal carcinomas (CRCs). Point mutations in codons 12, 13, and 61 are the most common *KRAS* mutations in CRC. There are few reports on insertions in *KRAS*, and little is known about its ability to activate the RAS pathway. The scarcity of data regarding insertion frequencies and nucleotide additions in *KRAS* impedes the management of patients with such mutations. We present data on *KRAS* insertions in CRC and discuss a case.
Materials and methods: Pyrosequencing and Sanger sequencing were performed to identify *KRAS* and *BRAF* mutations in paraffin-embedded samples of CRC. Expression of mismatch repair proteins was examined by immunohistochemistry.
Results: We detected a GGT insertion between codons 12 and 13 (c.36_37insGGT;p.G12_G13insG) in a CRC patient. We found that insertions in *KRAS* is very rare in CRC and that the most frequent type of insertion is c.36_37insGGT.
Conclusions: *KRAS* gene insertions represent a diagnostic and clinical challenge due to the difficult and unusual pyrosequencing findings and the lack of information regarding its clinical impact.

Core tip

KRAS mutation is a negative predictor of the response to anti-EGFR therapy in colorectal carcinoma. The most frequent and extensively studied mutation is found in codons 12, 13, and 61. Little is known about the impact of unusual *KRAS* mutations on treatment response or their impact on tissue morphology and clinical characteristics. Insertion type mutations can also be technically challenging to detect.

Introduction

KRAS mutations are found in 35% to 40% of colorectal carcinomas (CRCs) and are negative predictors of the response to anti-EGFR therapy (Lièvre et al., 2006). Such mutations can result from nucleotide exchange and deletion or insertion of DNA segments.

In CRC, most mutations in *KRAS* are single-base substitutions that result in a missense mutation that involves 1 codon, most commonly codons 12 and 13 (73% and 22%, respectively). The most frequent point mutations in codon 12 are GGT > GAT (c.35G > A; p.Gly12Asp) and GGT > GTT (c.35G > T p.Gly12Val), and that in codon 13 is GGC > GAC (c.38G > A; p.Gly13Asp) (Andreyev et al., 1998; Bos et al., 1987; Urosevic et al., 1993). Other less frequent missense mutations in *KRAS* have been reported in codons 61, 117, and 146 and other rare sites (Brink et al., 2003; Palmirotta et al., 2009). Single-base substitutions that involve more than 1 codon in the same tumor and the exchange of more than 1 base in the same codon have also been reported (Macedo et al., 2011).

The lack of experimental data on the pathogenicity of insertion-type mutations in *KRAS* and few findings regarding their frequency and nucleotide insertions impede the treatment of patients with such mutations. Insertions in *KRAS* have been reported in only 16 tumors.

We present data on insertions in *KRAS* and discuss a case of a GGT insertion between codons 12 and 13 of *KRAS* in a CRC patient with additional clinical and molecular findings.

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Materials and methods

Clinical history

A 65-year-old male underwent a colonoscopy due to anemia that was diagnosed in the preoperative exams for drainage of a hydrocephaly due to vascular encephalic infarction. He had a history of arterial systemic hypertension and diabetes. No gastrointestinal symptoms were present at the time of the colonoscopy. The colonoscopy showed a 7.0-cm stenosing mass on the right colon, with confirmation of the adenocarcinoma by biopsy. CEA levels were normal. The patient was subjected to thoracic, abdominal, and pelvic tomography staging exams, which were negative for metastatic lesions. A right colectomy resection was performed.

Histopathological findings

The tumor was a moderately differentiated invasive adenocarcinoma (Fig. 1A) with a mucinous component that constituted 5% of the tumor area, arising from a high-grade tubulovillous adenoma (Fig. 1B). Neoplastic cells had infiltrated the pericolonic subserous tissue, and the patient showed no vascular or perineural invasion. Sixty lymph nodes (LNs) were examined, all of which were negative for metastasis. Surgical margins were negative, and the small intestine mucosa and appendix were unremarkable with regard to microscopic alterations.

Per the AJCC 7th edition, the final pathological stage was pT3pN0pM0. The patient was classified as clinical stage II, and no adjuvant treatment was indicated, because there were no other high risk features of malignancy. After 12 months of follow-up, the patient did not experience recurrence of the tumor or metastasis.

Molecular pathology findings

The tumor was analyzed by immunohistochemistry to examine the loss of expression of gene repair machinery protein, due to its association with poor response to 5-fluorouracil-based treatments and as a prognostic marker (Jover et al., 2009) following an institutional workflow for all CRC specimens. Antibodies to MSH-2 (G219-1129-Cell Marque), MSH-6 (44-Ventana), MLH-1 (G168-728-Cell Marque), and PMS-2 (A16-4-BD) were with a streptavidin–biotin system. Internal and external positive controls worked properly.

Loss of expression was defined as lack of nuclear staining throughout the tumor and positive staining of non-neoplastic tissue on the same slide. In our case, all repair proteins were expressed.

KRAS mutation analysis was performed initially on 1 representative slide of the invasive tumor area. DNA was obtained by scraping neoplastic tissue from 5-um paraffin-embedded sections and extracted with a commercial kit (FFPE Qiagen). The DNA was sequenced by pyrosequencing reaction per manufacturer's instructions (*KRAS* Pyro KIT Q24, Qiagen). The pyrogram showed a GGT insertion in *KRAS* between codons 12 and 13 (c.36_37insGGT, p.G12_G13insG) (Fig. 1C). These findings were confirmed by standard Sanger reaction of the same DNA sample (Fig. 1D).

BRAF mutations in codon 600 were examined in the same DNA sample by pyrosequencing per manufacturer's instructions (*BRAF* Pyro KIT Q24, Qiagen); the tumor contained wild-type *BRAF*.

KRAS heterogeneity

To examine the intratumoral heterogeneity of the *KRAS* alteration, one different invasive neoplastic area than the first one examined and a preneoplastic adenomatous lesion were tested for the *KRAS* mutation by pyrosequencing; all areas had the same GGT insertion between

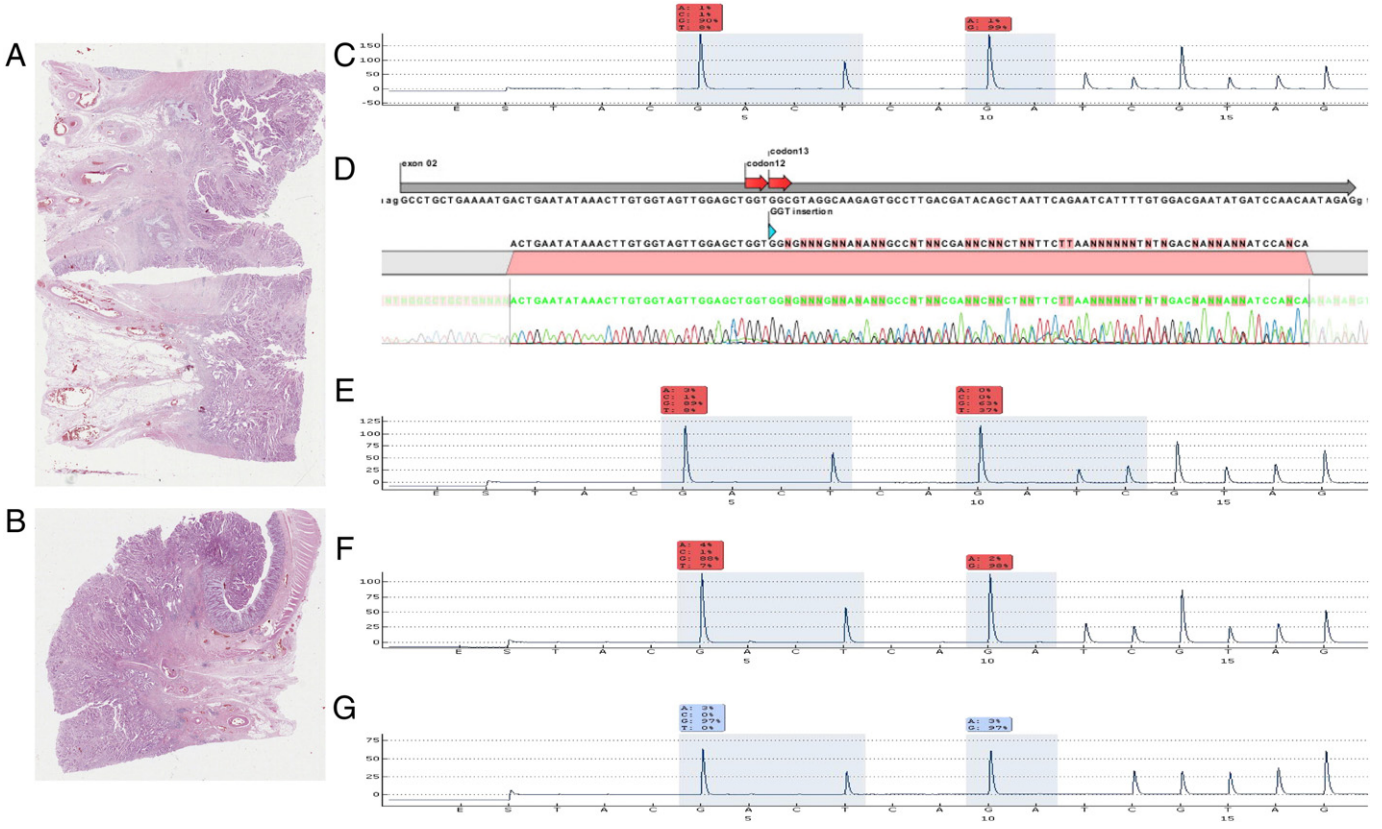

Fig. 1. A & B). Hematoxylin and eosin slides showing an invasive colorectal adenocarcinoma arising in an adenomatous lesion with high-grade dysplasia. The circled area indicates from where DNA was extracted. Pyrogram showing a GGT insertion between codons 12 and 13 of *KRAS* in an invasive area (C) with direct sequencing confirming the result (D). A distinct invasive area (E) and an adenomatous lesion (F) showing the same mutation. Pyrogram (G) showing wild-type *KRAS* in normal mucosa.