# *KRAS* mutation status impacts diagnosis and treatment decision in a patient with two colon tumours: a case report

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Received 28 July 2014
Revised 18 September 2014
Accepted 24 September 2014
Published Online First 13 October 2014

## ABSTRACT

*KRAS* mutation status predicts response to anti-EGFR therapy in colorectal cancer patients. Here we report an interesting case of discordant *KRAS* mutation status in a patient with two separate tumour foci. Tumour A in sigmoid colon invaded through muscularis propria into the subserosal fat with metastatic disease in regional lymph nodes (pT3N2b). Tumour B in ascending colon had a relatively lower stage and no metastasis (pT2N0). Both tumours showed similar morphology, immunohistochemical staining and microsatellite instability pattern. *KRAS* mutation, however, was detected only in tumour A. These findings indicate distinct clonal nature of these two tumours. The discordance of *KRAS* mutation status also suggests that a combination of anti-epidermal growth factor receptor and chemotherapy is likely the best treatment option for this patient. This case exemplifies a notion that comprehensive pathological work-up comprising molecular testing is critical to guide the diagnosis and treatment decisions for colorectal cancer patients with multiple tumours.

## INTRODUCTION

Colorectal cancer is the fourth leading cause of death worldwide causing approximately 608 000 deaths in 2008 and 8% of all cancer related deaths.[1] Besides surgery and traditional chemotherapy, anti-epidermal growth factor receptor (EGFR) antibodies such as cetuximab or panitumumab have routinely become treatment regimens either as a single agent or in combination with conventional chemotherapy for these patients.[2 3] However, patients with a colorectal tumour bearing a mutated *KRAS* gene fail to respond to anti-EGFR therapy.[4–6] *KRAS* mutations are present in approximately 30%–50% of colorectal tumours.[7] Testing for *KRAS* mutations is, therefore, recommended for colorectal cancer patients prior to the initiation of anti-EGFR therapy.[4–6 8]

Multiple tumours in colorectal cancer patients, either synchronous or metachronous, may be present as independent primaries or metastatic disease. In addition to histology and immunohistochemistry (IHC) staining, molecular testing is very useful in the pathology work-up of these cases.[9 10] This study presents an interesting case in which different *KRAS* mutation status is identified in a colorectal cancer patient with two separate tumours of similar morphology, IHC pattern and even identical microsatellite instability (MSI) status.

## CASE PRESENTATION

A patient with no family history of colon cancer presented for the follow-up of possible ulcerative colitis. Colonoscopy revealed a deep ulcer in the sigmoid colon and a single flat polyp in the ascending colon. Biopsies of each lesion were taken and adenocarcinoma was diagnosed in the specimen from the sigmoid colon, while malignancy could not be ruled out in the specimen from the ascending colon. A colectomy was performed and two tumours were identified during the procedure. The gross examination showed the first tumour (tumour A): a 4.7×3 cm, centrally ulcerated lesion with raised borders, located in the sigmoid colon; the second tumour (tumour B): a 1.5×1.4 cm, ulcerated lesion, located in the ascending colon. The distance between these two tumours was 96 cm.

Histologically, both tumours exhibited well to moderately differentiated with glandular to solid architecture, and pleomorphic nuclei with prominent nucleoli (figure 1). However, tumour A was classified to be of more advanced stage with deep infiltration through the muscularis propria and involving the subserosal fat tissue. Lymph-vascular invasion was noted and metastatic adenocarcinoma was identified in 8 out of 36 regional lymph nodes (pT3N2b). In contrast, tumour B extended to but not through the muscularis propria without lymph-vascular invasion. No metastasis was found in 27 regional lymph nodes (pT2N0). All the margins including proximal, distal, circumferential (radial) and vascular margin were negative for both tumours.

IHC was performed to further compare the morphology and investigate the nature of these two tumours. Tumour cells from both ascending and sigmoid colon showed a similar IHC pattern: weakly and focally positive for cytokeratin (CK)20, CDX-2, positive for MUC1, MUC3 and negative for CK7 and MUC2 in tumour A, with a weaker expression of CK 20 and CDX-2 in tumour B (table 1).

Molecular diagnostic tests were performed to provide insights to the pathological nature of the tumours. DNA was extracted and purified from paraffin embedded tissues obtained from these two tumours. The MSI analysis revealed identical MSI profiles in which both tumours A and B had four out of five tested microsatellite markers (D2S123, D17S250, D5S346 and BAT 26) stable and one microsatellite marker (BAT 25) unstable (table 2). These results indicate that both tumours A and B had low MSI (MSI-L). Additionally, *BRAF* and *EGFR* mutations were not detected in both

**To cite:** Li X, Pezeshkpour G, Phan RT. *J Clin Pathol* 2015;**68**:83–85.

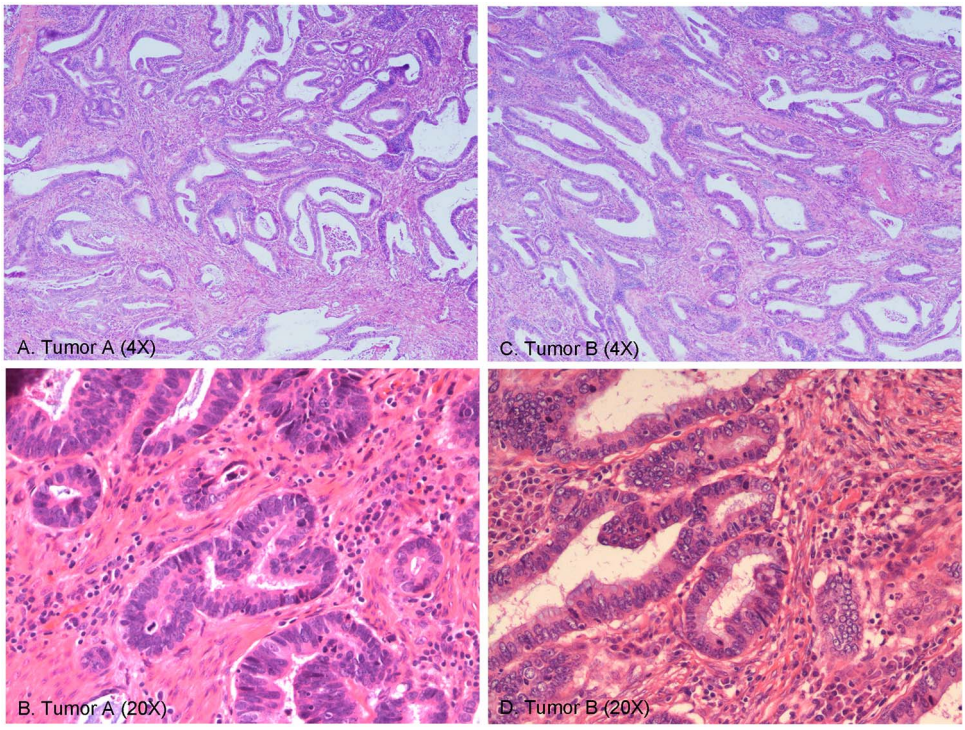


**Figure 1** H&E-stained sections of tumours: (A) tumour A (4×); (B) tumour A (20×); (C) tumour B (4×); and (D) tumour B (20×).

tumours A and B (data not shown). Molecular analysis, however, showed that a *KRAS* exon 2 point mutation (GGT→GAT) was detected in tumour A and its regional lymph nodes whereas none of tested *KRAS* mutations were detected in tumour B (table 3). This mutation (GGT→GAT) occurs at codon 13 of the *KRAS* gene, resulting in a single amino acid substitution, glycine to aspartic acid.

To further investigate a possibility that tumour B may contain a few clones with *KRAS* mutation that are not detected due to clonal dilution, tumour B paraffin section was partitioned into four different parts for microdissection. Each part had approximately 65%–85% tumour content. *KRAS* testing was performed by real-time PCR using Taqman probes specifically recognised *KRAS* wild-type and mutant alleles that can reliably detect *KRAS* mutation in as few as 5 mutated cells per 100 normal cells. The analysis revealed that no *KRAS* mutations are detected in all microdissected parts of tumour B (data not shown), confirming that the tumour B has no *KRAS* mutations.

## DISCUSSION

A patient with multiple synchronous or metachronous tumours may have independent primaries or metastatic disease. These individual tumours with identical histological and IHC pattern are found to show different molecular aberrations.[9] In the colon and rectum, incidence of multiple primary cancers is about 2%–5%[10] and the discordance of MSI status has been identified in individual tumours.[11] Accordingly, these tumours may possess different genetic profiles including a different *KRAS* mutation status.

The risk for ulcerative colitis associated colorectal cancer is increased at least 2-fold compared with the normal population. Colorectal cancer is observed in 5.5%–13.5% of all patients with ulcerative colitis.[12] These cancers typically can occur in the colon, are often multifocal[12] and have the same incidence of *KRAS* mutation in comparison with sporadic colorectal cancer.[13] In this study, both tumours A and B from this patient had a similar MSI profile (MSI-L). Together with the similarity of histological appearance and immunohistochemical pattern, these findings initially suggest that tumours A and B might have evolved from an identical tumour clone. Moreover, the tumour grade, stage and location also suggest that tumour B in ascending colon with relatively lower grade and stage is likely a metastasis from the more advanced tumour A in sigmoid, possibly through the lymphovascular spread.

A seminal study by Al-Mulla *et al*[14] has previously reported *KRAS* mutational heterogeneity in primary and secondary

**Table 1** Immunohistochemical staining in tumours A and B

| | Tumour A | Tumour B |
|---|---|---|
| CK7 | – | – |
| CK20 | + | $+^{w}$ |
| CDX-2 | + | $+^{w}$ |
| MUC1 | + | + |
| MUC2 | – | – |
| MUC3 | + | + |

$+^{w}$, weakly positive.

**Table 2** Microsatellite instability analysis in tumours A and B

| Microsatellites | Tumour A | Tumour B |
|---|---|---|
| D2S123 | Stable | Stable |
| D17S250 | Stable | Stable |
| D5S346 | Stable | Stable |
| BAT25 | Unstable | Unstable |
| BAT26 | Stable | Stable |

**Table 3** *KRAS* mutation analysis in tumours A and B

| Condon change | Tumour A | Tumour B |
|---|---|---|
| Gly12Val | – | – |
| Gly12Asp | – | – |
| Gly12Ser | – | – |
| Gly12Cys | – | – |
| Gly12Arg | – | – |
| Gly12Ala | – | – |
| Gly13Asp | + | – |
| Gly13Val | – | – |
| Gly13Ser | – | – |
| Gly13Arg | – | – |
| Gly13Ala | – | – |
| Gly13Cys | – | – |

colorectal carcinomas. Although the same *KRAS* mutations are found in the majority of patients with primary carcinomas and their metastases, many patients have a *KRAS* mutation in their primary carcinoma but none in liver metastases or patients have mutations in liver or lymph node metastases but none in the primary carcinoma,[14] indicating that *KRAS* mutations are not always essential for the metastatic potential or might occur at the later stages of tumour progression. Importantly, Al-Mulla *et al* also reported a patient who has multiple adenomas with different *KRAS* mutations but only one of these mutations is found in corresponding liver metastasis, suggesting that these adenomas are independent neoplasms.[14] In this context, the present case report is consistent with and in agreement with these findings. In fact, molecular analysis reveals that tumour A and metastatic regional lymph nodes contained a mutated *KRAS* gene whereas tumour B had a wild-type *KRAS* gene. This result, together with the findings of clear margins and no lymph-vascular invasion in tumour B, diminishes the possibility that tumour B is a metastasis from tumour A. Based on the patient's possible history of ulcerative colitis, these findings support the notion that these two tumours most likely originated from distinct clones and that both tumours possibly developed synchronously, with tumour A displaying a more advanced stage.

It is possible that the tumour B may evolve later to have the same *KRAS* mutation found in tumour A at a later stage of tumour progression. The present analysis, however, has no direct evidence to support this possibility.

This patient likely needs adjuvant therapy besides surgery due to the high tumour stage (pT3N2b). If only tumour A had been sampled for molecular testing, a *KRAS* positive result would exclude this patient from anti-EGFR therapy. As a result, tumour B would lose the benefit of anti-EGFR therapy. Likewise, if only tumour B had been tested and showed that it has *KRAS* wild-type, the patient would have been treated with an anti-EGFR regimen. Tumour A, carrying a *KRAS* mutation, would have failed to respond this treatment.[4 15] Unsuccessful outcome can be anticipated in either two scenarios. Instead, a combination of traditional chemotherapy and anti-EGFR therapy is likely the best treatment option with the best consequent prognosis for this patient.[3]

## CONCLUSIONS

Traditional methods of histology and IHC are often insufficient to accurately anticipate the nature of the individual tumours in colorectal cancer patients with multiple primaries. This study demonstrates the importance of a comprehensive pathological work-up comprising molecular testing that impacts the diagnosis and guides treatment decisions.

### Take home message

Molecular diagnostic tools for all the tumour sites provide insight to the pathological nature of the tumours and present a profound effect in the treatment decisions.

**Acknowledgements** We would like to thank Dr Faramarz Naeim and Dr Priyadashini Kumar for their critical review and comments on our manuscript.

**Contributors** XL, GP and RTP designed the study and performed the data analysis. XL and RTP organised the data for publication. XL and RTP wrote the manuscript.

**Competing interests** None.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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