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Association between KRAS G13D mutations and anastomotic recurrence in colorectal cancer

Two case reports

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Abstract

Rationale: The prevalence of anastomotic recurrence (AR) in colorectal cancer (CRC) after resection of the primary tumor (PT) is 5% to 14%. However, no association has been observed between specific somatic genetic alterations and AR. Such associations may shed light on the mechanism of AR.

Patient concerns: We experienced 2 patients with AR of CRC. The first patient was a 42-year-old woman who underwent resection of an AR of rectal cancer 2 times within 19 months after resection of the PT. The second patient was a 77-year-old woman who underwent resection of an AR of ascending colon cancer twice within 38 months after resection of the PT.

Diagnosis: Both cases were diagnosed as repetitive AR.

Interventions: Loss of heterozygosity analysis, microsatellite instability (MSI) study of 9 microsatellite loci, and mutational analysis of *KRAS*, *BRAF*, *APC*, *TP53*, and *SMAD4* were performed.

Outcomes: All the lesions, except 1, harbored mutations in *APC, KRAS,* and *TP53*, without MSI, after neoadjuvant chemoradiotherapy. The *APC, KRAS,* and *TP53* mutations were pathogenic or likely pathogenic in the PTs and ARs. Both women harbored the same KRAS G13D mutation, which accounts for 8% of all *KRAS* mutations in sporadic CRC s. The probability of the incidental occurrence of KRAS G13D mutations in both cases is 0.64%, provided that the mutations were independent of AR.

Lessons: Our findings may shed light on the mechanism of AR in CRC, namely, that the PT harbored the same mutations as the AR and the lesions in both cases harbored the KRAS G13D mutation.

Abbreviations: AR = anastomotic recurrence, CIN = chromosomal instability, CRC = colorectal cancer, CRT = chemoradiotherapy, CT = computed tomography, DST = double-stapling technique, LOH = loss of heterozygosity, MSI = microsatellite instability, MSS = microsatellite stable, PCR = polymerase chain reaction, PT = primary tumor, WT = wild type.

Keywords: anastomotic recurrence, colorectal neoplasms, genetic alterations, KRAS G13D

1. Introduction

Anastomotic recurrence (AR) is a form of local recurrence in colorectal cancer (CRC). The incidence of AR is reportedly 5% to 14%,[1–3] despite the performance of rectal lavage and the acquisition of sufficient surgical margin, as well as other preventive measures.

There are 2 major hypotheses regarding the pathogenesis of AR. One pertains to the implantation of cancer cells in the suture line, and the other to the metachronous development of another primary tumor (PT) in an area of proliferative instability.[4,5] However, the precise mechanisms and causes of AR remain unclear. Another possible hypothesis is that cancer cells are biologically more aggressive and adhesive to the bare area of the anastomotic site in the case of AR. To the best of our knowledge, only 2 reports to date have investigated the genomic profile of PTs and Ars.[6,7] Costi et al investigated the microsatellite instability (MSI) and loss of heterozygosity (LOH) at 7 sites (*Myc-L, BAT26, BAT40, D5S346, D18S452, D18S64,* and *D16S402*) in the PTs and ARs of 18 patients.[6] They observed LOH at 5q21 and/or 18p11.23 in both the PT and AR in more than half of the cases; this result was similar to that observed in the control group. Vakini et al used next-generation sequencing to determine the mutational status of cancer-associated genes. They reported that the PT and AR shared between 50% and 100% of the mutations in 13 microsatellite stable (MSS) lesions.[7] No specific somatic genetic alterations have been identified in association with AR to date. Herein, we report 2 cases of repetitive AR with somatic genetic analyses.

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2. Case presentation

2.1. Case 1

A 42-year-old woman visited the hospital with complaints of rectal bleeding, nausea, and left abdominal pain. Colonoscopy revealed a full circumferential cancerous lesion (1-PT) in the recto-sigmoid colon. High anterior resection using the double-stapling technique (DST) was performed. Pathological examinations showed a moderately differentiated adenocarcinoma (T3, N1b, M0) according to the Union for International Cancer Control tumor-node-metastasis classification. After surgery, the patient received 8 cycles of capecitabine plus oxaliplatin adjuvant therapy. Eight months after the initial surgery, the patient experienced rectal bleeding. Colonoscopy revealed AR (1-AR1) of a semi-circumferential tumor at the anastomotic site. Local resection of the AR was performed using the DST. The pathological examination showed a moderately differentiated adenocarcinoma (T3, N0, M0), which was compatible with AR. Nine months after the second surgery, which corresponded to 17 months after the initial surgery, the patient again experienced rectal bleeding, and colonoscopy revealed another recurrence of a circumferential tumor (1-AR2) at the anastomotic site. Preoperative chemoradiotherapy (CRT) was performed with a regimen of tegafur–uracil, leucovorin, and CPT-11 and concurrent external-beam radiotherapy of a total dose of 50.4 Gy (1.8 Gy per day). Hartmann's procedure, total abdominal hysterectomy, and bilateral salpingo-oophorectomy were performed. Pathological examination showed a moderately differentiated adenocarcinoma (T4b [uterus invasion], N0, M0). Twenty-seven months after the initial surgery and 5 months after the third surgery, the patient underwent a computed tomography (CT) scan, which revealed lung and bone metastases and local recurrence in the pelvis. Although the patient received FOLFIRI, she died due to disease progression 11 months after the third surgery.

2.2. Case 2

A 77-year-old woman with ascending colon cancer (2-PT) underwent right hemicolectomy with a functional end-to-end anastomosis at another hospital. Pathological examination revealed a moderately differentiated adenocarcinoma (T3, N1b, M0). Three years after the initial surgery, colonoscopy revealed tumor recurrence at the anastomotic site. It was diagnosed as AR (2-AR1), and surgery was performed. During the surgery, paraaortic lymph node metastasis was suspected, and local resection of the anastomotic site with side-to-end anastomosis was performed along with paraaortic lymph node dissection. Pathological examination showed a moderately differentiated adenocarcinoma (T3, N2b, M1a [paraaortic lymph node]). The histopathology of the recurring lesions resembled that of the PT.

Four years after the initial surgery (1 year after the second surgery), colonoscopy revealed a recurrent lesion at the anastomotic site and another recurrence at the stump of the side-to-end anastomosis. The patient received a CT scan, which showed no evidence of distant metastases. A second AR (2-AR2) was diagnosed. Local resection of the anastomotic site with side-to-end anastomosis was performed. Pathological examinations showed that the recurrent lesions were both moderately differentiated adenocarcinomas (T2, N1a, M0). After the surgery, the patient received a regimen of tegafur–uracil as adjuvant therapy.

Five months after the third surgery, lung metastases were detected by a whole-body CT scan. Resection of the lung metastases was performed, and postoperative follow-up was performed on an outpatient basis. The lung metastasis recurred 1 year and 8 months after resection. Because the patient was old and we did not want to administer FOLFOX, FOLFIRI, or molecularly targeted agents due to the increased risk of toxicity, tegafur–uracil was administered. However, the patient died due to disease progression 3 years and 8 months after the third operation.

2.3. DNA and RNA extraction

Tumor tissues and corresponding normal mucosae were obtained from the surgically resected specimens, and were either snap frozen in liquid nitrogen immediately after resection and stored at −80°C or immersed in RNA*later* Tissue Protect Tubes (Qiagen, Valencia, CA) overnight at 4°C, followed by storage at −20°C until use. Genomic DNA was extracted from the frozen tissue samples using the QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions, or from formalin-fixed, paraffin-embedded tumor samples using the TaKaRa DEXPAT® Easy kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. Total RNA was extracted using Trizol reagent (Invitrogen, ThermoFisher Scientific, Carlsbad, CA), and treated with DNase I (Takara Bio) according to the manufacturer's instructions.

2.4. Microsatellite instability and LOH analysis

Using the extracted DNA, we analyzed the MSI and LOH status through polymerase chain reaction (PCR) using AmpliTaq Gold DNA polymerase (Applied Biosystems, ThermoFisher Scientific, Foster City, CA) at 9 microsatellite loci: *BAT25, BAT26, D2S123, D5S346, D17S250, TP53, D18S46, D18S363,* and *D18S474.*

We previously reported that 18q is a prognostic marker for CRC.[8] We defined LOH at each locus as a 50% reduction in the height of one of the 2 allele peaks in the tumor DNA relative to the non-neoplastic control DNA. We also investigated the LOH ratio, which is known to predict the worst survival for CRC.[9]

We defined the LOH ratio (%) as

$$\text{LOH ratio}(\%) = \frac{\text{Total number of chromosomes with LOH}}{\text{Total number of chromosomes that could be evaluated for LOH}} \times 100$$

to evaluate the severity of chromosomal instability (CIN) based on the actual frequency of LOH among the evaluable loci. A LOH ratio ≥33% were classified as CIN high and an LOH ratio <33% was classified as CIN low.[9]

2.5. Mutation analysis

We examined the mutational status using Sanger sequencing. Using extracted DNA or complementary DNA prepared from the DNase I-treated RNA and PrimeScript RT master mix (Takara Bio), we performed PCR using PrimeSTAR GXL DNA polymerase (Takara Bio) according to the manufacturer's instructions. We designed primers for *KRAS* (codons 12, 13, 59, 61, 117, and 146), *BRAF* (codon 600), *APC* (codons 1282–1581), *TP53* (codons 1-345), and *SMAD4* (codons 1-170 and 350-553) using Primer3Plus (https://primer3plus.com/). The amplified PCR products were cleaned up using ExoSAP-IT® (Applied Biosystems), cycle sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and analysis was conducted using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). If any mutation was present, we analyzed whether the mutation was pathogenic using the Catalogue of Somatic Mutations in Cancer (COSMIC) (Sanger Institute, UK) or ClinVar (NCBI) databases.

2.6. Clinicopathological features of the PTs and ARs

The clinicopathological features of the PTs and ARs are shown in Table 1. The histopathological diagnoses of the PT and recurrent specimens were concordant (moderately differentiated adenocarcinoma) in both Case 1 and Case 2. Both cases were positive for lymph node metastasis at the time of PT resection. The colonoscopy findings and clinical course of each of the cases are summarized in Figure 1.

2.7. Microsatellite instability and LOH analysis

The results of the analysis for the 9 microsatellite markers are shown in Table 2. Both patients showed an MSS status in the PT and ARs. In Case 1, we observed LOH at the 5q and 18q loci in the PT (1-PT) and the first AR (1-AR1). However, it was not observed in the second AR (1-AR2). In Case 2, LOH was observed only at 17p in the first AR (2-AR1). PT specimens were not available. LOH was not observed in the second AR (2-AR2).

We also evaluated the LOH ratio for each tumor. In Case 1, the LOH ratio of the PT (1-PT) and first AR (1-AR1) was 50% (CIN high). However, that of the second AR (1-AR2) was 0% (CIN low). In Case 2, the LOH ratios of the first AR (2-AR1) and second AR (2-AR2) were both 0% (CIN low).

2.8. Mutation analysis

The results of sequence analyses of *KRAS*, *BRAF*, *APC*, *TP53*, and *SMAD4* are shown in Table 3.

In Case 1, the PT (1-PT) and first AR (1-AR1) shared KRAS (p.G13D), APC (p.E1353*), and p53 (p.V272L) mutations. The *BRAF* and *SMAD4* genotypes in the indicated codons were wild type (WT) in both the tumors. However, there were no mutations in the second AR (1-AR2). The KRAS (p.G13D) and APC (p.E1353*) mutations were pathogenic, according to COSMIC. The p53 (p.V272L) mutation was also likely pathogenic.

In Case 2, the first AR (2-AR1) and second AR (2-AR2) shared the KRAS (p.G13D) mutation. In the second AR (2-AR2), both the tumors shared the APC (p.L1488fs) and p53 (p.P152L) mutations. In both the tumors, the *BRAF* and *SMAD4* genotypes in the indicated codons were WT. The KRAS (p.G13D), APC (p.L1488fs), and p53 (p.P152L) mutations were pathogenic, according to COSMIC. The PT (2-PT) specimens were not available.

2.9. Ethical review

This study was approved by the Ethics Committee of the University of Tokyo (No. G3552-(3)).

Informed written consent was obtained from the patients for publication of this case report and accompanying images.

3. Discussion and conclusions

We reported 2 cases of repetitive CRC AR with genetic analyses. In both cases, all the lesions except for 1, which was discovered after neoadjuvant CRT, harbored *APC*, *KRAS*, and *TP53* mutations without MSI; this probably corresponds to the consensus molecular subtype 3.[10] Interestingly, the tumors shared the same KRAS G13D mutation, which accounts for 8% of all *KRAS* mutations in sporadic CRC. The probability of the incidental occurrence of KRAS G13D mutations in both cases is 0.64%, provided that KRAS G13D mutations are independent of AR. The relationship between *KRAS* mutational patterns and AR development has not been reported before. Few studies have investigated the genetic alterations associated with AR in CRC,[6,7] and our findings may shed light on the mechanism of AR in such cases.

All the lesions except the lesion resected after adjuvant CRT (1-AR2) showed a good chemoradiation effect, and the sample may have contained fewer cancer cells and a higher number of normal cells, resulting in the negative results of the genetic analysis. This finding was in line with a previous report by Vakini et al,[7] who performed next-generation sequencing of 36 tumors from 14 patients and defined the mutational status of 341 cancer-associated genes. They demonstrated that the lesions displayed an

Table 1

Clinicopathological features of the primary tumors and anastomotic recurrences.

Case/Age (yr)/Sex	Number of recurrences	Tumor location	Anastomotic technique	Interval after initial surgery	Treatment	Histological diagnosis of the primary tumor and recurrent specimen
1/42/F		Rectosigmoid colon	DST		High anterior resection	Moderately differentiated adenocarcinoma–T3, N1b, M0
	1	Anastomosis	DST	8 months	Resection of the anastomosis	Moderately differentiated adenocarcinoma–T3, N0, M0
	2	Anastomosis	(–)	1 year, 6 months	Hartmann's operation after neoadjuvant chemoradiotherapy	Moderately differentiated adenocarcinoma, T4b (uterus invasion)–N0, M0, Circumferential resection margin positive
277/F		Ascending colon	FEEA		Right hemicolectomy	Moderately differentiated adenocarcinoma–T3, N1b, M0
	1	Anastomosis	Side-to-end anastomosis	3 years	Resection of the anastomosis	Moderately differentiated adenocarcinoma–T3, N2b, M1a (paraaortic lymph node)
	2	Stump	Side-to-end anastomosis	4 years	Resection of the anastomosis	Moderately differentiated adenocarcinoma–T2, N1a, M0
		Anastomosis				Moderately differentiated adenocarcinoma–T2, N1a, M0

DST = double-stapling technique, FEEA = functional end-to-end anastomosis.

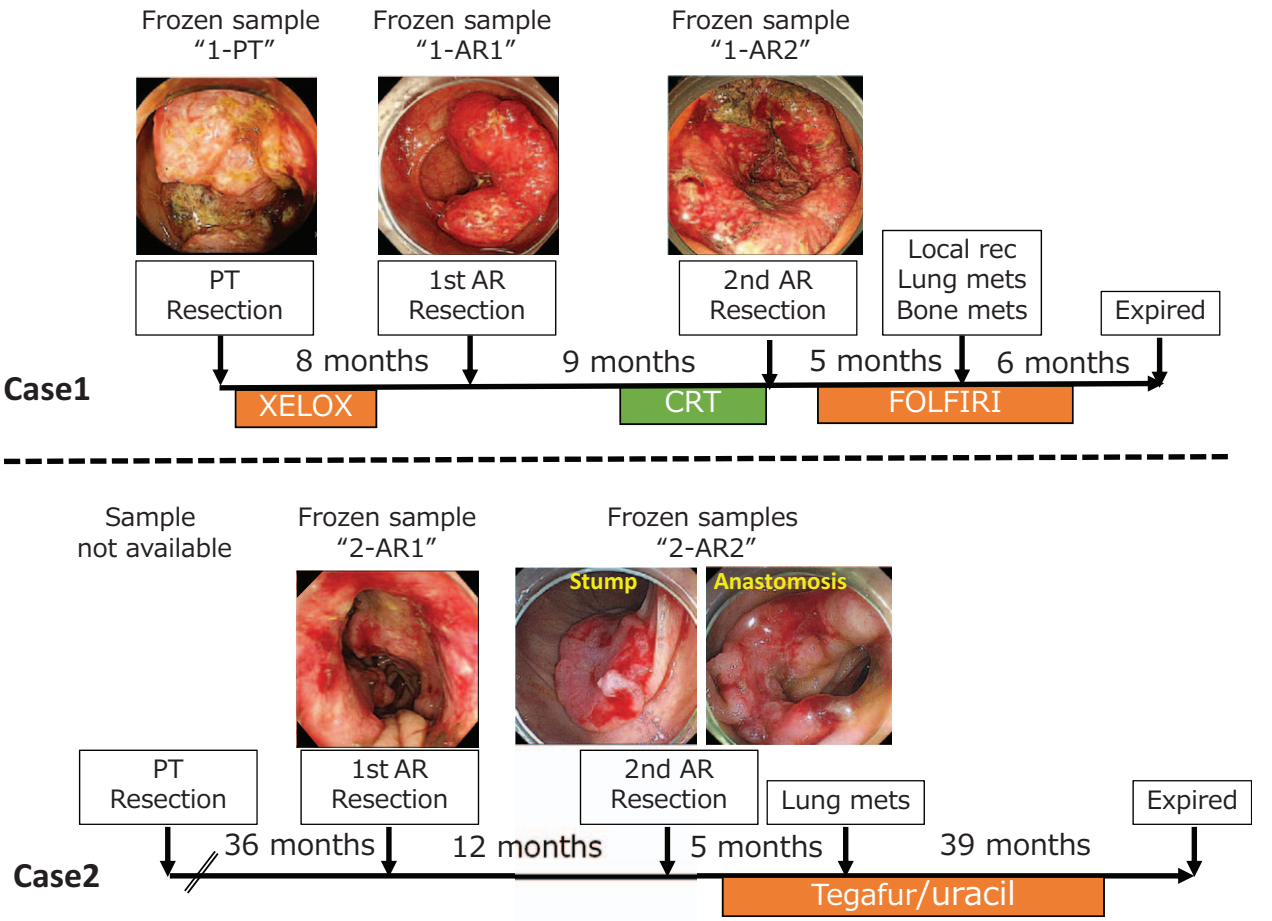

Figure 1. Colonoscopy findings and clinical course of the 2 cases. Case 1: a 42-year-old woman with rectal cancer. Case 2: a 77-year-old woman with ascending colon cancer.

MSS pattern in 13 patients and had a higher *KRAS* mutation rate (69%), without reaching significance. Unfortunately, the authors did not present detailed information on *KRAS* mutational patterns, such as codon positions and amino acid substitution patterns. Costi et al performed MSI and LOH analyses of 7 sites (*Myc-L, BAT26, BAT40, D5S346, D18S452, D18S64,* and *D16S402*) in the PTs and ARs of 18 patients, and demonstrated that more than half of the cases had lesions with CIN.[6]

The mutation pattern of *KRAS* was concordant in terms of amino acid substitution (G13D) in both cases in the present study. The reported incidence of *KRAS* mutation is approximately 40% among all colorectal tumors.[11] *KRAS* mutations frequently occur at codon 12 (G12D, 13%; G12V, 9%) or 13 (G13D, 8%). The probability of the incidental occurrence of KRAS G13D mutations in both cases is 0.64%, which is too rare for it to be independent of AR. Therefore, we hypothesized that

Table 2

Molecular alterations in the primary colon cancer and anastomotic recurrences.

				Microsatellite markers								
				BAT25	BAT26	D2S123	D5S346	D17S250	TP53	D18S46	D18S363	D18S474
				Gene locus								
Case	Sample	Tumor location	Template	4q	2p	2p	5q	17p	17p	18q	18q	18q
				c-kit	hMSH2	hMSH2,6	APC	BRCA1	TP53	SMAD4	DCC	SMAD4
1	PT	Rectosigmoid colon	Frozen/gDNA	MSS	MSS	RET	LOH	RET	N.I.	LOH	LOH	LOH
	AR1	Anastomosis	Frozen/gDNA	MSS	MSS	RET	LOH	RET	N.I.	LOH	LOH	LOH
	AR2	Anastomosis	Frozen/gDNA	MSS	MSS	RET	RET	RET	N.I.	RET	RET	RET
2	PT	Ascending colon		–	–	–	–	–	–	–	–	–
	AR1	Anastomosis	FFPE/gDNA	MSS	MSS	N.I.	N.I.	RET	N.D.	N.I.	N.D.	RET
	AR2	Stump	Frozen/gDNA	MSS	MSS	N.I.	N.I.	RET	RET	N.I.	RET	RET
		Anastomosis	Frozen/gDNA	MSS	MSS	N.I.	N.I.	RET	RET	N.I.	RET	RET

AR = anastomotic recurrence, FFPE = formalin-fixed, paraffin-embedded, GDNA = genomic DNA, LOH = loss of heterozygosity, MSS = microsatellite stable, N.D. = not determined; –, Not performed, N.I. = not informative, PT = primary tumor, RET = retention of heterozygosity.

Table 3

Mutations in the primary tumor and anastomotic recurrences.

Case	Sample	Tumor location	Template	*KRAS* Codons 12, 13, 59, 61, 117, 146	*BRAF* Codon 600	*APC* Codons 1282–1581	*TP53* Codons 1–345	*SMAD4* Codons 1–170, 350–553
1	PT	Rectosigmoid colon	Frozen/gDNA	–	WT	–	–	–
			cDNA	G13D	–	E1353*	V272L	WT
	AR1	Anastomosis	Frozen/gDNA	–	WT	–	–	–
			cDNA	G13D	–	E1353*	V272L	WT
	AR2	Anastomosis	Frozen/gDNA	–	WT	–	–	–
			cDNA	WT	–	WT	WT	WT
2	PT	Ascending colon		–	–	–	–	–
	AR1	Anastomosis	FFPE/gDNA	G13D	WT	–	–	–
	AR2	Stump	Frozen/gDNA	–	WT	–	–	–
			cDNA	G13D	–	pL1488fs	P152L	WT
		Anastomosis	Frozen/gDNA	–	WT	–	–	–
			cDNA	G13D	–	pL1488fs	P152L	WT

AR = anastomotic recurrence, cDNA = complementary DNA, FFPE = formalin-fixed paraffin-embedded, GDNA = genomic DNA, PT = primary tumor, WT = wild type; –, not performed.

the KRAS G13D mutation may be associated with AR due to the aggressive nature of the cancer cells. A study reported that patients with KRAS G13D-mutant tumors showed poor prognosis with standard chemotherapy alone or best supportive care.[12] They also showed improved outcomes under treatment with cetuximab.[13] This is a paradox, considering the reports that *KRAS*-mutant tumors are resistant to anti-epidermal growth factor receptor therapy.[12] Another study also supported this result in that KRAS G13D mutations were associated with improved clinical outcomes when cetuximab was added to first-line chemotherapy.[14] Our patients may have benefitted from chemotherapy with cetuximab, although current guidelines do not recommend a cetuximab regimen for *KRAS*-mutant CRC, and thus health insurance in Japan does not cover this regimen. Several studies have reported a correlation between colorectal liver metastasis and *KRAS* mutation.[15,16] However, no reports have demonstrated a relationship between AR and *KRAS* mutation. Further studies with larger sample sizes are warranted.

The terminology of AR is arguable, and distinguishing between AR and secondary PT development may be difficult. Gopalan et al reviewed the literature and defined local recurrences and secondary PTs according to the duration between the development of anastomotic tumors and PTs.[4] However, this definition is arbitrary. More theoretically, secondary PTs harbor distinct genetic mutations, whereas the mutations of an AR may be similar to those of the PT. In our cases, the lesions shared the same mutation profile, and genetically they were considered ARs.

There are several limitations to this study. First, the PT specimen in Case 2 (2-PT) was unavailable. In addition, the second AR specimen in Case 1 (1-AR2) could not yield sufficient DNA due to the chemoradiation effect. The present study investigated only 2 cases, and thus the number of lesions assessed was limited. However, despite the limited number of AR cases, we were able to clarify the genetic profile and determine potential relationships between genetic abnormalities and AR. Second, we did not perform comprehensive next-generation sequencing and only included key gene profiles. There may be novel mutations associated with AR.

Despite such limitations, the present case reports may provide some insights on the mechanism of AR in CRC, in that they showed that the PT harbored the same mutations as the AR, and the lesions in both cases harbored the KRAS G13D mutation.

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References

[1] Bowne WB, Lee B, Wong WD, et al. Operative salvage for locoregional recurrent colon cancer after curative resection: an analysis of 100 cases. Dis Colon Rectum 2005;48:897–909.
[2] Read TE, Mutch MG, Chang BW, et al. Locoregional recurrence and survival after curative resection of adenocarcinoma of the colon. J Am Coll Surg 2002;195:33–40.
[3] de Chaisemartin C, Penna C, Goere D, et al. Presentation and prognosis of local recurrence after total mesorectal excision. Colorectal Dis 2009;11:60–6.
[4] Gopalan S, Bose JC, Periasamy S. Anastomotic recurrence of colon cancer-is it a local recurrence, a second primary, or a metastatic disease (local manifestation of systemic disease). Indian J Surg 2015;77:232–6.
[5] Kim MY, Oskarsson T, Acharyya S, et al. Tumor self-seeding by circulating cancer cells. Cell 2009;139:1315–26.
[6] Costi R, Santi C, Bottarelli L, et al. Anastomotic recurrence of colon cancer: genetic analysis challenges the widely held theories of cancerous cells' intraluminal implantation and metachronous carcinogenesis. J Surg Oncol 2016;114:228–36.
[7] Vakiani E, Shah RH, Berger MF, et al. Local recurrences at the anastomotic area are clonally related to the primary tumor in sporadic colorectal carcinoma. Oncotarget 2017;8:42487–94.

[8] Watanabe T, Wu TT, Catalano PJ, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med 2001;344:1196–206.
[9] Watanabe T, Kobunai T, Yamamoto Y, et al. Chromosomal instability (CIN) phenotype, CIN high or CIN low, predicts survival for colorectal cancer. J Clin Oncol 2012;30:2256–64.
[10] Dienstmann R, Vermeulen L, Guinney J, et al. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat Rev Cancer 2017;17:79–92.
[11] Normanno N, Tejpar S, Morgillo F, et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. Nat Rev Clin Oncol 2009;6:519–27.
[12] Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408–17.
[13] De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p. G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 2010;304: 1812–20.
[14] Tejpar S, Celik I, Schlichting M, et al. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. J Clin Oncol 2012;30:3570–7.
[15] Margonis GA, Kim Y, Spolverato G, et al. Association between specific mutations in KRAS codon 12 and colorectal liver metastasis. JAMA Surg 2015;150:722–9.
[16] Margonis GA, Kim Y, Sasaki K, et al. Codon 13 KRAS mutation predicts patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. Cancer 2016;122:2698–707.