

Colorectal cancer in cases of multiple primary cancers: Clinical features of 59 cases and point mutation analyses

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Abstract. The present study aimed to investigate the occurrence and clinical features of cases of multiple primary cancers including colorectal cancer (MPCC). The medical records of patients with colorectal cancer (CRC) who underwent surgery at the Third Xiangya Hospital of Central South University (Changsha, China) between August 2007 and August 2014 were retrospectively analyzed. Patients with MPCCs were identified and mutation analyses were performed on colon specimens. The results revealed that among 1,311 patients with CRC, 59 had MPCC (including 35 cases of ≥ 1 CRC with ≥ 1 other cancer type, and 24 cases with multiple CRCs and no other primary cancers). Foci occurred on the right side of the colon (n=32), in the rectum (n=28), and on the left side of the colon (n=24). MPCCs were synchronous in 24 patients, metachronous in 32 patients, and both in 3 patients. Age of onset and presence of polyps were identified as significantly different between MPCC and CRC overall (P<0.05); however, sex or adenoma incidence were not observed to differ significantly between

groups. Mutation incidence rates in 26 specimens were 11.54% for KRAS proto-oncogene GTPase (KRAS) G13D, 3.85% for KRAS Q61R and 3.85% B-Raf proto-oncogene serine/threonine kinase V600E. Mutations of exon 21 of the epithelial growth factor receptor gene, including L858R and L861Q, and of KRAS G12V were not detected. In conclusion, the likelihood of occurrence of MPCC is closely associated with the age of onset and the presence of polyp(s). Routine examination of multiple systems is necessary for patients with CRC to avoid missed diagnosis and misdiagnosis. Further study is required to demonstrate the molecular mechanism of CRC in cases of multiple primary cancers.

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumor types in humans. The annual incidence of CRC worldwide is >1,000,000 cases, which is greater than the incidence of all other cancer types, with the exception of lung and breast cancer (1). In 2011, there were 310,244 new cases of CRC in China, accounting for 9.2% of all new cases of cancer; additionally, there were 149,722 mortalities due to CRC in 2011, accounting for 7.09% of all cancer-associated mortalities (2). Evidence indicates that the incidence of CRC has increased each year between 1998 and 2007 and has continued to increase thereafter (3). It has long been recognized that the development of CRC is associated with the accumulation of certain genetic mutations, including components of the epidermal growth factor receptor (EGFR) signaling pathway (4). Evidence has also suggested that mutations in KRAS proto-oncogene GTPase (KRAS) and B-Raf proto-oncogene serine/threonine kinase (BRAF) are associated with a worse patient prognosis in CRC (5). Therefore, detection of EGFR, KRAS and BRAF mutations is important in studies of CRC.

The term 'multiple primary cancers' refers to cases in which independent primary malignant tumors occur simultaneously or successively in one or multiple organs in the same individual. Based on cancer registry records from between 1986 and 1995 at the Cancer Institute Hospital of The Japanese Foundation for Cancer Research (Tokyo, Japan), Ueno *et al* (6) reported

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Abbreviations: CRC, colorectal cancer; MPCC, multiple primary cancers including ≥ 1 colorectal cancer; CCOC, colorectal cancer with other primary cancer type; MCC, multiple CRCs and no other primary cancer types; EGFR, epidermal growth factor receptor; KRAS, KRAS proto-oncogene GTPase; BRAF, B-Raf proto-oncogene serine/threonine kinase

Key words: colorectal cancer, multiple primary colorectal cancers, multiple colorectal cancers, synchronous cancer, metachronous cancer

that among 24,498 patients with tumors, 1,281 (5.2%) had multiple primary cancers, and out of 1,587 cases of CRC, there were 142 (8.9%) cases of multiple primary cancer. Therefore, increasing the understanding of multiple primary cancers and CRCs, and strengthening the screening of patients for whom CRC is suspected is expected to greatly reduce the rate of misdiagnosis and missed diagnosis, provide novel strategies for treatment and thus improve the prognosis for CRC patients.

The purpose of the present study was to investigate the pathogenesis of cases of multiple primary cancer that included ≥ 1 CRC (MPCC) and provide evidence to aid the prevention and treatment of CRC, including in cases of MPCC.

Subjects and methods

Patients. A total of 1,311 patients who received surgical treatment for CRC at the Third Xiangya Hospital of Central South University (Changsha, China) between August 2007 and August 2014 were included in this study. Patient data was obtained from medical records and is presented in Table I. All patients had signed informed consent forms and the study received approval from Xiangya Hospital Ethics Committee. A subgroup of these patients was confirmed by pathological and/or cytological examinations to have MPCC.

Group inclusion and exclusion criteria

CRC group. Patients were required to have pathologically confirmed CRC and to previously have received surgical treatment at the Third Xiangya Hospital. Patients with metastatic or recurrent cancers, and those whose cancers were not pathologically confirmed, were excluded.

CRC with other cancer (CCOC) group. Patients were selected according to the modification of diagnostic criteria from Warren and Gates (7) in combination with clinical observations: i) The patient must have multiple primary cancers including ≥ 1 CRC and ≥ 1 primary cancer at another location; ii) each primary tumor must be pathologically confirmed to be malignant and have its own specific pathological morphology; iii) the possibility of the subsequent tumors having arisen from the first primary tumor via recurrence or metastasis must be excluded.

Multiple CRCs (MCC) group. Patients were selected according to the diagnostic criteria of Lee *et al* (8) in combination with clinical observations: i) The patient must have ≥ 2 primary cancers in the colon/rectum, with no primary tumors at other sites, and each tumor must be pathologically confirmed to be malignant; ii) cancer foci must be independent of each other, with a transitional zone composed of heterotypic cell glands between tumor foci and the normal intestinal wall; iii) the possibility of metastasis, recurrence and submucosal diffusion of the primary cancer must be excluded; iv) there must be no recurrence of previous surgical anastomotic stoma, and new foci must be clearly distinct from previous surgical incisions; v) patients must not have been diagnosed with familial adenoma or enteropathic arthritis-induced cancer.

DNA isolation and mutation assessment. Hematoxylin and eosin staining was implemented to confirm that specimens contained $>80\%$ cancer cells, and areas enriched with

Table I. Clinical epidemiological characteristics of 1,311 colorectal cancer patients following surgery.

Variable	n	%
Sex		
Male	761	58.00
Female	550	42.00
Age, years		
≤ 35	63	4.80
36-59	600	45.77
≥ 60	648	49.43
Pathological type		
Tubular adenocarcinoma	1,143	86.98
Mucinous adenocarcinoma	89	6.77
Signet-ring cell carcinoma	6	0.46
Other	76	5.79
Lesion site		
Rectum	669	50.11
Left side of the colon	278	20.83
Right side of the colon	388	29.06

malignant cells were identified prior to DNA extraction by two independent pathologists. DNA was extracted from formalin-fixed, paraffin-embedded tissue specimens using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at -80°C until use.

Mutation hotspots in *EGFR* (L858R and L861Q), *KRAS* (G12V, G13D and Q61R) and *BRAF* (V600E) were detected. Polymerase chain reaction (PCR) amplification was performed as follows: 1 min of initial denaturation at 95°C ; 35 cycles of amplification consisting of 30 sec at 94°C , 40 sec at 57°C and 30 sec at 72°C , with a final additional elongation at 72°C for 7 min. Used primers are as follows: *EGFR* L858R/L861Q forward primer, 5'-CCAGGAACG TACTGGTGA AAA-3'; *EGFR* L858R/L861Q reverse primer, 5'-TGACCTAAAGCCACCTCCTT-3'; *KRAS12/13* forward primer, 5'-TATAAGGCCTGCTGAAAATGACTG-3'; *KRAS12/13* reverse primer, 5'-TATTCGTCCACAAA TGATTCTGA-3'; *KRAS61* forward primer, 5'-AATTGATGGAGAAACCTGTCTCTT-3'; *KRAS61* reverse primer, 5'-TCCTCATGTACTGGTCCCTCATT-3'; *BRAF* V600E forward primer, 5'-CTTCATAATGCTTGCTCTGATAGG-3'; *BRAF* V600E reverse primer, 5'-GCATCTCAGGGCCAA AAATT-3'. Non-template control was included in each batch during the experiment, and sequencing was performed using ABI 3500xL Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

Statistical analysis. The medical records of the overall population of patients with CRC and the subset of patients with MPCC were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Numerical data were analyzed with the χ^2 test. Measurement data are presented as the mean \pm standard deviation. The two-sample t-test was used for comparisons

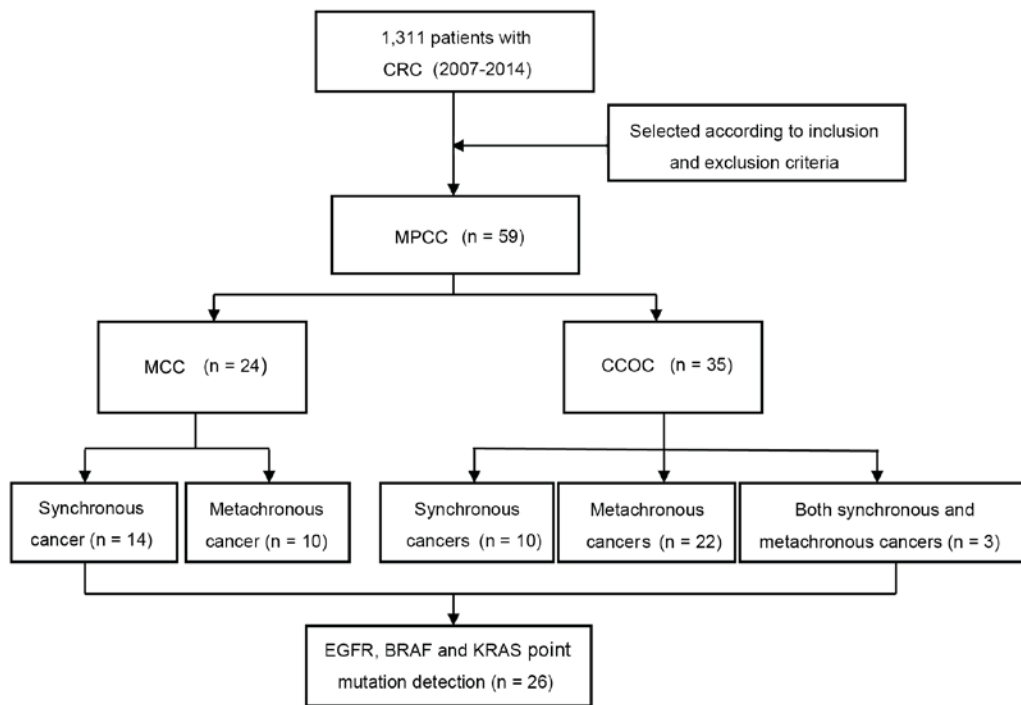


Figure 1. Flowchart of patient selection. CRC, colorectal cancer; MPCC, multiple primary cancers including CRC; MCC, multiple CRCs; CCOC, CRC and other primary cancer; EGFR, epidermal growth factor receptor; BRAF, B-Raf proto-oncogene serine/threonine kinase; KRAS, KRAS proto-oncogene GTPase.

between groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient selection. A flow diagram illustrating the selection of patients in the study is presented in Fig. 1. A total of 1,311 patients underwent surgery for CRC during the study period. Of these, 59 patients had MPCC (including 35 with CCOC and 24 with MCC).

Clinical epidemiological characteristics of CRC patients following surgery. The clinical epidemiological characteristics of the 1,311 patients who underwent CRC surgery are presented in Table I. The overall patient population consisted of 761 males and 550 females (male:female ratio, 1.38:1), with a mean age of 58.36 ± 13.07 years (range, 20-91 years).

Patients were divided into three age groups: Young (≤ 35 years), middle-aged (36-59 years), and elderly (≥ 60 years). In the young group, 47.62% of patients had rectal cancer, 23.81% had cancer of the right side of the colon, and 28.57% had cancer of the left side of the colon. In the middle-aged group, 54.17% of patients had rectal cancer, 20.00% had cancer of the left side of the colon, and 25.83% had cancer of the right side of the colon. In the elderly group, 46.91% of patients had rectal cancer, 20.68% had cancer of the left side of the colon, and 32.41% had cancer of the right side of the colon.

Among the total study population, 1,143 (86.98%) patients had tubular adenocarcinoma, 89 (6.77%) had mucinous adenocarcinoma, 6 (0.46%) had signet-ring cell cancer, and 76 (5.79%) had undifferentiated carcinoma or other cancer types. For cases in which the tumors were of the same

histological type, the highest-level pathological diagnosis was included in the statistics. Of the total 1,311 patients, 3 patients had different pathological types between two CRC foci and therefore were retrospectively included in the statistical analysis. A significant difference in pathological type between age groups was identified ($P < 0.05$; Fig. 2).

Comparisons of age of onset, sex and pathological types between patients with MPCC and CRC. There was a significant difference in the age of onset between the 59 patients with MPCC and the 1,311 patients with CRC ($P < 0.05$); however, no significant difference was observed in sex between them ($P > 0.05$). Among the 1,311 patients with CRC, 318 (24.265%) had adenoma and 446 (34.02%) had polyp(s). By comparison, 19 (30.20%) of the 59 patients with MPCC had adenoma and 33 (55.93%) had polyps. There was no significant difference between the CRC and MPCC cohorts with regard to the proportion of patients with adenoma ($P = 0.166$); however, a significant difference was observed in the proportions of patients with polyp(s) ($P = 0.001$; Table II).

Occurrence and clinical features of patients with MPCC. According to the inclusion criteria, 59 (4.50%) patients, consisting of 33 (55.93%) males and 26 (44.07%) females with a mean age of 62.11 ± 11.74 years (range, 40-81 years) had MPCC. Among these patients, 56 patients (94.92%) had double cancer, 2 (3.39%) had triple cancer, and 1 (1.69%) had quadruple cancer (Fig. 3A).

There were 122 primary cancer foci among the 59 patients with MPCC. These foci were located in the large intestine [$n = 84$; including the rectum ($n = 28$), left side of the colon ($n = 24$), and right side of the colon ($n = 32$)], small intestine ($n = 6$),

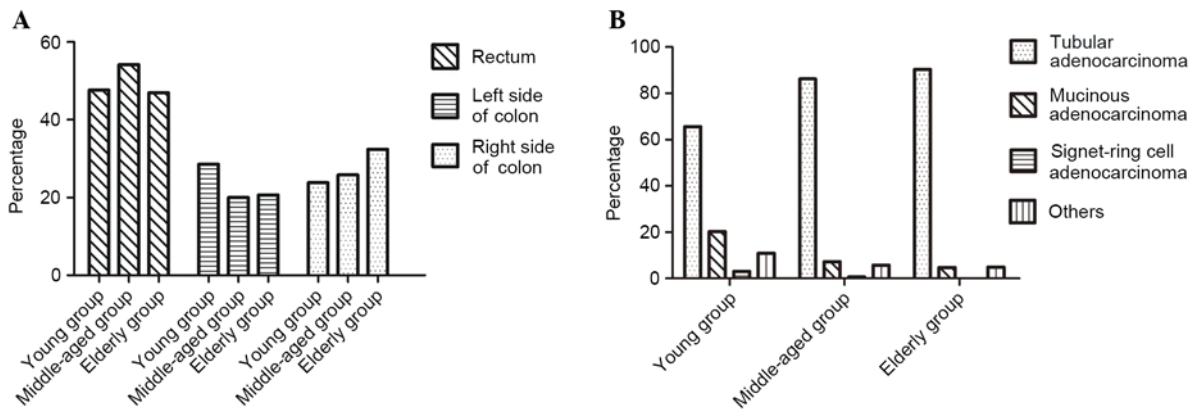


Figure 2. Characteristics of lesions in CRC patients of different ages. (A) Constituent ratios of lesion locations in CRC patients of different ages. (B) Pathological types of CRC in patients of different ages.

Table II. Comparison of 1,311 patients with CRC and 59 patients with MPCC.

Variable	MPCC	All CRC	P-value
Total, n	59	1,311	-
Age, years			0.035
Mean \pm SD	62.11 \pm 11.74	58.36 \pm 13.07	
Range	40-81	20-91	
Sex			0.747
Male	33	761	
Female	26	550	
Complication			
Adenoma	19	318	0.166
Polyp(s)	33	446	0.001

CRC, colorectal cancer; MPCC, multiple primary cancers including CRC; SD, standard deviation.

ovary (n=3), uterus (n=3), cervix (n=3), mammary gland (n=3), kidney (n=3), bladder (n=3), nasopharynx (n=2), lung (n=2), stomach (n=2), ureter (n=2), prostate (n=2), esophagus (n=1), thyroid gland (n=1), liver (n=1) and the left iliac fossa (n=1; Fig. 3B).

As well as the 59 pathologically confirmed primary CRC foci (i.e. 1 per patient) included in the cohort, the 63 additional primary cancer foci were located in the digestive system [n=35; including the large intestine (n=25), small intestine (n=6), stomach (n=2), esophagus (n=1), and liver (n=1)], reproductive system [n=14; including the mammary gland (n=3), ovary (n=3), uterus (n=3), cervix (n=3), and prostate (n=2)], urinary system [n=8; including the kidney (n=3), bladder (n=3), and ureter (n=2)], respiratory system [n=4; including the nasopharynx (n=2), and lung (n=2)], endocrine system (n=1) and hematological system (n=1).

Of the 59 patients with MPCC, 24 (1.83% of total CRC cohort) patients had synchronous cancer, 32 (2.44% of total CRC cohort) patients had metachronous cancer, and 3 (0.23% of total CRC cohort) patients had both. There was 1 patient with quadruple cancer, including both synchronous and

metachronous cancers, comprising 2 CRCs, 1 cervical cancer and 1 endometrial cancer (Fig. 3C).

Among the 24 patients with synchronous cancer, 18 patients (75.00%) were diagnosed with a synchronous cancer simultaneously with the diagnosis of CRC (before or during the CRC surgery), and 2 patients were diagnosed with the second cancer ~1 month after surgery. A total of 6 patients (25.00%) in the synchronous cancer group were misdiagnosed.

Among the 32 patients with metachronous cancer, the mean disease onset interval (between two cancer diagnoses) was 49.25 months (range, 7 months to 20 years). For the 10 patients with metachronous cancers in the MCC group, the mean disease onset interval was 23.80 months (range, 7 months to 6 years). In the 22 patients with metachronous cancers in the CCOC group, the mean disease onset interval was 60.86 months (range, 7 months to 20 years; Table III).

Detection of point mutations. The common CRC-associated point mutation loci of the *EGFR*, *KRAS* and *BRAF* genes were analyzed in available specimens from 26 patients with MPCC. Mutations of *KRAS* G12V and in exon 21 of the *EGFR* gene (including L858R and L861Q) were not detected. The incidence rates were 11.54% for *KRAS* G13D, 3.85% for *KRAS* Q61R and 3.85% for *BRAF* V600E (Table IV).

Discussion

The incidence of CRC has increased in recent years, and rectal cancers account for a larger proportion than colon cancers (9). In the present study, the lesions in middle-aged patients predominantly occurred in the rectum, whereas, in elderly patients, lesions most commonly occurred in the colon; cancer of the right side of the colon was more common than the left side in elderly patients. These results are consistent with previous reports (10,11). Adenocarcinoma is the predominant type of CRC; with age, patients possess an increasing risk of adenocarcinoma overall, whereas the risk of signet-ring cell carcinoma or mucinous adenocarcinoma decreases (12).

There is evidence that 17-19% of patients with new tumors have previously experienced malignant tumors (13,14). Multiple cancers of the head and neck are among the most extensively

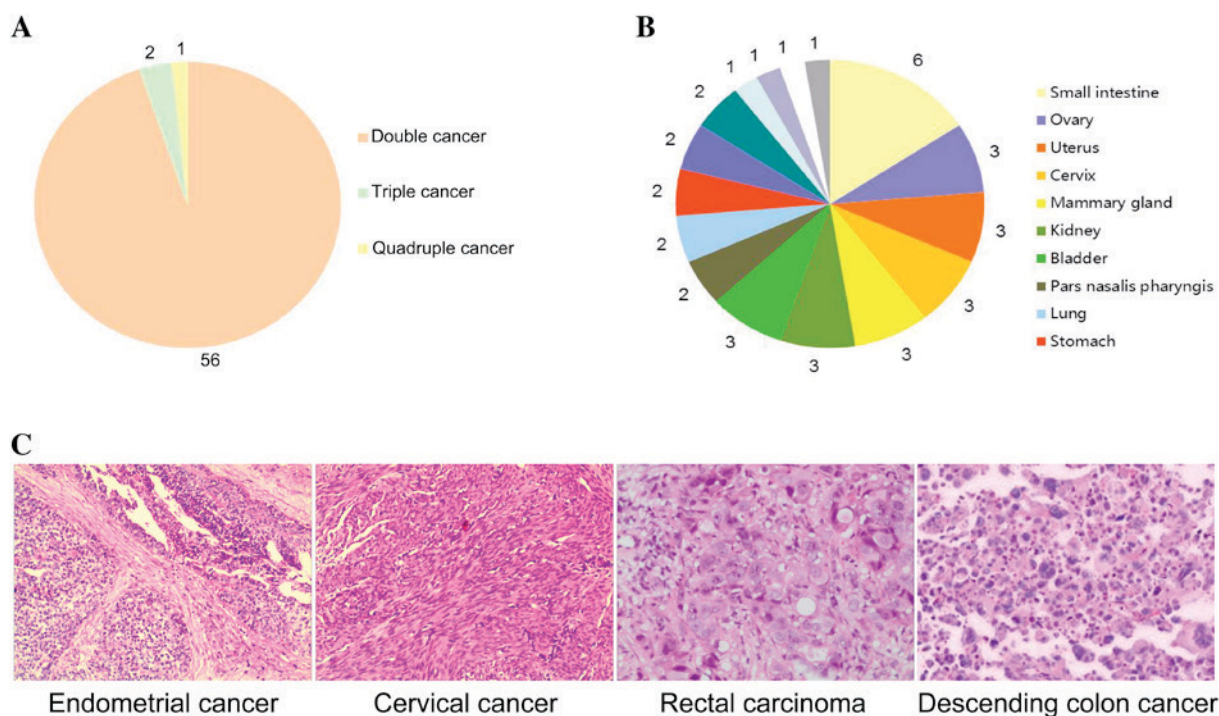


Figure 3. Occurrence and clinical characteristics of 59 cases of multiple primary cancers with CRC. (A) Numbers of double, triple and quadruple cancer cases; values indicate absolute numbers of cases. (B) Locations of 38 extracolonic primary malignant lesions in 35 patients with CRC and ≥ 1 other type of primary cancer; values indicate absolute numbers of lesions. (C) Histological images (hematoxylin and eosin staining; magnification, $\times 100$) from one patient with quadruple cancers, synchronous and metachronous, including two CRCs. CRC, colorectal cancer.

Table III. Intervals between onset of each cancer in 32 patients with metachronous multiple primary cancers including CRC.

Group	Total, n	Disease onset interval, years					Mean interval, months
		<1	≥ 1 -<3	≥ 3 -<5	≥ 5 -<10	≥ 10	
MCC	10	5 (50)	4 (40)	0 (0)	1 (10)	0 (0)	23.80
CCOC	22	5 (23)	6 (27)	4 (18)	4 (18)	3 (14)	60.86
Total	32	10 (31)	10 (31)	4 (13)	5 (16)	3 (9)	49.25

Data are presented as number of patients (%). CRC, colorectal cancer; CCOC, CRC and other primary cancer; MCC, multiple CRCs and no other primary cancer types.

studied of recent years (15,16). A small number of studies have reported on multiple CRCs and other multiple primary cancers. The results of the present study indicate that the incidence of MPCC among CRC cases is 4.50%, which is consistent with the incidence reported in a previous series of studies (6,17-19). There was a significant difference in the age of onset between the 59 patients with MPCC and the 1,311 patients with CRC ($P < 0.05$). A significant difference in the number of cases complicated by polyp(s) was identified between the 59 patients with MPCC and the overall population of 1,311 patients with CRC ($P < 0.05$), indicating that the occurrence of MPCC is associated with the presence of polyp(s) (20,21).

Among the 59 patients with MPCC, the proportion of males ($n=33$; 55.93%) was higher than that of females ($n=26$; 44.07%), which is similar to the incidence rates of single primary CRC in males and females (22). Among the patients with MPCC, the proportion with metachronous cancers

was greater than the proportion with synchronous cancers. Among patients with MPCC, double cancer was the most common type, and triple or greater multiples of cancers were rarely identified. Metachronous cancers predominantly occurred within 1-3 years, followed by within 5 years, after the diagnosis of CRC. In addition to the 59 primary foci in the large intestine, the other primary foci were mainly located in the digestive system, reproductive system, urinary system and respiratory system. This is consistent with the field cancerization theory (23); specifically, as the esophagus and gastrointestinal tract are the passage through which food passes, the lungs are the air channels, and the urinary system is the fluid waste channel, all of these organs are particularly exposed to carcinogens in food or air.

It is well known that the occurrence of cancer is closely associated with the accumulation of gene mutations, and patients with incipient cancer thus possess a high risk for

Table IV. Point mutations detected among 26 specimens from patients with multiple primary cancers including a colorectal cancer.

Gene and mutation locus	Incidence of mutation	
	n	%
<i>EGFR</i> L858R	0	0.00
<i>EGFR</i> L861Q	0	0.00
<i>KRAS</i> G12V	0	0.00
<i>KRAS</i> G13D	3	11.54
<i>KRAS</i> Q61R	1	3.85
<i>BRAF</i> V600E	1	3.85

EGFR, epidermal growth factor receptor; *KRAS*, *KRAS* proto-oncogene GTPase; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase.

secondary cancer (24,25). In this study, common point mutation loci of the *EGFR*, *KRAS* and *BRAF* genes in 26 specimens from patients with multiple cancers were analyzed. The rates of incidence were 11.54% for *KRAS* G13D and 3.85% for *KRAS* Q61R mutations, which were evidently lower than previously reported rates of 30–60% (5,26,27). The incidence rate of *BRAF* V600E mutation was 3.85%, which was also lower than previously reported (12.44%) (26). There is evidence that survival following surgery in patients who developed metachronous MPCCs tended to be better than that of patients with a single carcinoma (6). This suggests that the mutation profile of MPCC is likely to be associated with its prognosis. This hypothesis will be investigated further in a larger study by this group.

The findings of the present study suggest that, for patients with CRC, detailed medical records, physical examination and associated auxiliary examinations are required, and pathological examination of living tissue is necessary to determine whether a new foci is a primary cancer, metastatic cancer or recurrent cancer. Thus, treatment strategies can be developed with the purpose of lengthening the patient's survival time and improving their quality of life. Studies in patients with MPCC aid the development of novel strategies for investigating the occurrence and underlying mechanism of the development of this disease, and identifying molecular targets for its treatment.

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