Association between somatic *RET* mutations and clinical and genetic characteristics in patients with metastatic colorectal cancer

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Research

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Abstract

Background

Rearranged during transfection (*RET*) is a targetable oncogene. *RET* fusions have been reported in patients with metastatic colorectal cancer (mCRC). However, *RET* mutations in mCRC are less studied. Here, we aimed to characterize the clinical, pathological, and molecular landscape of *RET*-mutated mCRC.

Methods

Five hundred and eighty-two patients were included in this study. Next-generation sequencing was performed to detect *RET* mutations and calculate tumor mutation burden (TMB). We compared the clinical, pathological, and molecular characteristics of mCRC cases with tumors that harbored somatic *RET* mutations ($N = 16$, 2.7%) or had wild-type *RET* ($N = 566$, 97.3%).

Results

Males comprised the absolute majority of cases with *RET* mutations (15/16 [93.8%]) compared to their fraction among cases with wild-type *RET* (339/566 [59.9%]). Furthermore, all patients with *RET* mutations were younger than 60 years (16/16 [100%]), whereas such patients were less predominant in the group with wild-type *RET* (379/566 [67.0%]). Individuals with tumors positive for *RET* mutations more frequently exhibited mucinous histology (5/16 [31.2%] versus 55/566 [9.7%]), exhibited a lower incidence of liver metastasis (4/16 [25.0%] versus 335/566 [59.2%]) and higher incidence of peritoneal metastasis (9/16 [56.2%] versus 161/566 [28.4%]), expressed wild-type *TP53* (8/16 [50.0%] versus 120/566 [21.2%]), and showed an increased frequency of MSI-high (6/16 [37.5%] versus 18/566 [3.2%]). In those with microsatellite-stable mCRC, patients with *RET* mutations had a higher median TMB than patients with wild-type *RET* (9.4 versus 6.7 mutations/Mb, respectively, $P = 0.001$). The median progression-free survival was similar in individuals with mutated and wild-type *RET* on the oxaliplatin-based regimen (7.1 versus 8.7 months, $P = 0.516$).

Conclusions

Our study suggests that cases with *RET* mutations represent a separate mCRC subtype. Further studies are needed to evaluate the efficacy of RET inhibitors in mCRC patients with *RET* mutations.

Background

The *RET* (rearranged during transfection) gene encodes a receptor tyrosine kinase that plays a key role in the activation of MAPK, PI3K, JAK, and PKA/C pathways [1]. Aberrant RET activation is involved in various types of tumorigenesis, including medullary thyroid cancer (MTC), non-small cell lung cancer

(NSCLC), and colorectal cancer (CRC) [2–4]. Thus, RET has emerged as a therapeutic target in patients with aberrant RET activation. Two selective RET inhibitors, selpercatinib (LOXO-292) and pralsetinib (BLU-667), have shown promising anti-cancer activities and are currently widely evaluated in clinical trials [2, 5–8].

There are mainly two mechanisms underlying *RET* oncogenic activation. The first is chromosomal rearrangement, which causes fusion of RET with a partner protein [9, 10]. The second mechanism is somatic or germline gain-of-function mutation [11]. *RET* fusions were observed in a small fraction (< 1%) of metastatic CRC (mCRC), where their presence was associated with old age, right-sided tumor origin, wild-type *RAS* and *BRAF*, microsatellite instability (MSI)-high tumors, and poor prognosis [12]. However, the prevalence as well as clinical, pathological, and molecular features of somatic *RET* mutations in patients with mCRC are largely unknown. Kato et al. examined a cohort of 300 patients with CRC and identified two individuals with *RET* mutations [13]. Such rare occurrence of *RET* mutations precluded their further analysis. Hence, this study aimed to evaluate the frequency and phenotypic characteristics of mCRC with somatic *RET* mutation in a larger patient cohort.

Methods

The following patients were selected: (1) those diagnosed with mCRC based on pathology samples at our institute between January 1, 2015 and March 31, 2020; (2) those who had an available record of the next-generation sequencing result performed at our institute. Those who (1) accepted chemotherapy or radiotherapy before next-generation sequencing analysis or (2) had no available follow-up information were excluded. The study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center, and all patients provided informed consent. The detailed description of the methods for next-generation sequencing and tumor mutation burden (TMB) calculation is provided in the Supplementary Material.

We evaluated the association between the presence of somatic *RET* mutations and the following variables: age, gender, primary tumor location, differentiation, mucinous histology, T stage, N stage, time to metastasis, metastatic organs, *TP53* mutations, *APC* mutation, *RAS* mutations, *BRAF* mutations, *SMAD4* mutations, *PIK3CA* mutations, *ERBB2* amplifications, and MSI status. Patients' characteristics were compared by using the chi-squared test. TMBs were compared by using the Kruskal-Wallis test. Kaplan-Meier survival analysis and log-rank test were performed to detect differences in progression-free survival (PFS). The statistical tests were two-tailed and the differences were considered statistically significant when $P < 0.05$.

Results

Five hundred and eighty-two patients were included in this study (Fig. 1). The patients' median age was 54 (range, 17–88) years, and 354 patients (60.8%) were male. Somatic *RET* mutations were identified in 16 (2.7%) patients. The genomic alterations of *RET*-mutated tumors are shown in Table 1 and Fig. 2.

Table 1
Genomic Alterations in *RET*-Mutated Metastatic Colorectal Cancer

Patient ID	Genomic alterations
1	<i>RET</i> , EX3, R177W; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSS
2	<i>RET</i> , EX3, R133C; <i>KRAS</i> A146T; <i>BRAF</i> wild-type; MSS
3	<i>RET</i> , EX5, V351I; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSI-high
4	<i>RET</i> , EX6, N359T; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSS
5	<i>RET</i> , EX7, T451M; <i>KRAS</i> G12D; <i>BRAF</i> wild-type; MSS
6	<i>RET</i> , EX7, V485L; <i>NRAS</i> Q61L; <i>BRAF</i> wild-type; MSI-high
7	<i>RET</i> , EX10, E616del; <i>KRAS</i> G12D; <i>BRAF</i> wild-type; MSI-high
8	<i>RET</i> , EX10, E623G; <i>KRAS</i> G13D; <i>BRAF</i> wild-type; MSS
9	<i>RET</i> , EX11, V642F; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSS
10	<i>RET</i> , EX11, K662M; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSS
11	<i>RET</i> , EX11, P695S; <i>KRAS</i> G13D; <i>BRAF</i> wild-type; MSI-high
12	<i>RET</i> , EX11, V706M; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSS
13	<i>RET</i> , EX15, V871I; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSI-high
14	<i>RET</i> , EX15, V871I; <i>KRAS</i> G13D; <i>BRAF</i> wild-type; MSI-high
15	<i>RET</i> , EX15, S891L; <i>RAS</i> wild-type; <i>BRAF</i> wild-type; MSS
16	<i>RET</i> , EX15, S891L; <i>RAS</i> wild-type; <i>BRAF</i> K601E; MSS
MSI-high: microsatellite instability -high; MSS: microsatellite stable.	

The presence of *RET* mutations was associated with male sex (15/16 [93.8%] patients with mutated *RET* vs. 339/566 [59.9%] patients with wild-type *RET*), younger age (all 16 patients with mutated *RET* [100%] were $\leq$ 60 years old vs. 379 out of 566 [67.0%] patients with wild-type *RET*), mucinous histology (5/16 [31.2%] patients with mutated *RET* vs. 55/566 [9.7%] patients with wild-type *RET*), less liver metastasis (4/16 [25.0%] patients with mutated *RET* vs. 335/566 [59.2%] patients with wild-type *RET*), and more peritoneal metastasis (9/16 [56.2%] patients with mutated *RET* vs. 161/566 [28.4%] patients with wild-type *RET*, Table 2). Nine (12.3%) out of 73 male patients who were younger than 60 years of age and had peritoneal metastasis harbored *RET* mutations.

Table 2
Characteristics of Patients with *RET*-Mutated Metastatic Colorectal Cancer

Characteristics	<i>RET</i> ^{mutation} , <i>N</i> (%)	<i>RET</i> ^{wild-type} , <i>N</i> (%)	<i>P</i>
Gender			0.007
Male	15 (93.8)	339 (59.9)	
Female	1 (6.2)	227 (40.1)	
Age			0.002
>60	0 (0)	187 (33.0)	
≤60	16 (100)	379 (67.0)	
Primary tumor location			0.603
Right colon	6 (37.5)	162 (28.6)	
Left colon	6 (37.5)	198 (35.0)	
Rectum	4 (25.0)	206 (36.4)	
Differentiation			0.784
Well / moderate	11 (68.8)	416 (73.5)	
Poor	5 (31.2)	150 (26.5)	
Mucinous histology			0.018
Yes	5 (31.2)	55 (9.7)	
No	11 (68.8)	511 (90.3)	
T stage			0.415
1	0 (0)	2 (0.4)	
2	0 (0)	21 (3.7)	
3	5 (31.2)	266 (47.0)	
4	7 (43.8)	161 (28.4)	
N stage			0.029
0	6 (37.5)	123 (21.7)	
1	0 (0)	164 (29.0)	
2	5 (31.2)	146 (25.8)	
Time to metastasis			0.269

Characteristics	<i>RET</i> ^{mutation} , <i>N</i> (%)	<i>RET</i> ^{wild-type} , <i>N</i> (%)	<i>P</i>
Synchronous	9 (56.2)	398 (70.3)	0.009
Metachronous	7 (43.8)	168 (29.7)	
Liver metastasis			
Yes	4 (25.0)	335 (59.2)	0.257
No	12 (75.0)	231 (40.8)	
Lung metastasis			
Yes	2 (12.5)	159 (28.1)	0.024
No	14 (87.5)	407 (71.9)	
Peritoneal metastasis			
Yes	9 (56.2)	161 (28.4)	0.452
No	7 (43.8)	405 (71.6)	
Distant nodes metastasis			
Yes	3 (18.8)	73 (12.9)	0.923
No	13 (81.2)	493 (87.1)	

As shown in Table 3, *RET* mutations were more frequent in patients with wild-type *TP53* and MSI-high tumors (6/16 [37.5%] patients with mutated *RET* vs. 18/566 [3.2%] patients with wild-type *RET*). There were no significant associations between *RET* mutation presence and *APC*, *RAS*, *BRAF*, *SMAD4*, *PIK3CA*, or *ERBB2* status. In patients with microsatellite-stable mCRC, those with *RET* mutations had a higher TMB than those with wild-type *RET* (median TMB 9.4 and 6.7 Muts/Mb respectively, *P*= 0.001). In patients with MSI-high mCRC, those with *RET* mutations had a TMB similar to that of patients with wild-type *RET* (median TMB 66.6 and 59.2 Muts/Mb, respectively, *P*= 0.923).

Table 3
Association between the Presence of *RET* Mutation and Other Molecular Characteristics

Characteristics	<i>RET</i> ^{mutation} , <i>N</i> (%)	<i>RET</i> ^{wild-type} , <i>N</i> (%)	<i>P</i>
<i>TP53</i>			0.012
Wild-type	8 (50.0)	120 (21.2)	
Mutated	8 (50.0)	446 (78.8)	
<i>APC</i>			0.595
Wild-type	4 (25.0)	194 (34.3)	
Mutated	12 (75.0)	372 (65.7)	
<i>RAS</i>			0.622
Wild-type	9 (56.2)	280 (49.5)	
Mutated	7 (43.8)	286 (50.5)	
<i>BRAF</i>			0.319
Wild-type	15 (93.8)	503 (88.9)	
V600E mutation	0 (0)	49 (8.7)	
No V600E mutation	1 (6.2)	14 (2.4)	
<i>SMAD4</i>			0.500
Wild-type	12 (75.0)	468 (82.7)	
Mutated	4 (25.0)	98 (17.3)	
<i>PIK3CA</i>			0.721
Wild-type	13 (81.2)	481 (85.0)	
Mutated	3 (18.8)	85 (15.0)	
<i>ERBB2</i>			1.000
Amplification	16 (100)	548 (96.8)	
No amplification	0 (0)	18 (3.2)	
MSI status			< 0.001
MSS	10 (62.5)	548 (96.8)	
MSI-high	6 (37.5)	18 (3.2)	
MSI-high: microsatellite instability -high; MSS: microsatellite stable.			

The majority of the studied patients (413/582, 71.0%) were administered oxaliplatin-based first-line chemotherapy (FOLFOX or XELOX), whereas 63 (10.8%) received irinotecan-based chemotherapy (FOLFIRI or XELIRI). Among the mCRC patients on the oxaliplatin-based regimen, PFS was similar between patients with *RET* mutations and those with wild-type *RET* (median PFS 7.1 vs. 8.7 months, respectively, $P = 0.516$, Fig. 3).

Discussion

In our analysis of 582 patients with mCRC, we detected *RET* mutations in 16 (2.7%) individuals. Furthermore, *RET* mutations were more often present in younger male patients and they were associated more strongly with mucinous histology, weaker liver metastasis, and stronger peritoneal metastasis. Given that the incidence of *RET* mutations in mCRC patients is low, our study provides valuable information to potentially help physicians identify the enriched subgroups in which *RET* mutations could be more frequent.

Kato et al. studied *RET* aberrations in 4,871 patients with diverse malignancies [13]. Three hundred patients with CRC were included, and only two of them (0.7%) had *RET* mutations. The tumor stages were unknown, and this might explain the low mutation rate of *RET* in that study. Recently, Zhao et al. retrospectively reviewed 3,272 patients with CRC and documented that the *RET* mutation rate was 3.39% [11], which was similar to the rate observed in the present study. The additional information regarding the association of *RET* mutations with other factors (sex, age, histology, and metastasis sites) revealed in our study suggests a potential enrichment strategy for further trials with targeted agents focused on the *RET* mutation.

To date, there are several commercially available inhibitors demonstrating activity against RET. Most of them are non-specific inhibitors, such as cabozantinib, vandetanib, lenvatinib, sorafenib, sunitinib, and alectinib [14]. Recently, two selective RET inhibitors have been introduced to the clinic. Pralsetinib has been demonstrated to inhibit the growth of tumors driven by various *RET* mutations and fusions *in vivo*, and it showed durable clinical responses in patients with *RET*-altered NSCLC [15, 16]. Selpercatinib also showed promising activity in patients with *RET*-fused NSCLC and *RET*-fused or mutated MTC [7, 8, 14, 17]. Two patients with *RET*-mutated mCRC were included in a clinical trial (NCT03037385), and the efficacy of pralsetinib is still under evaluation in these two patients.

RET fusions have been observed in a small subgroup of patients with mCRC. *RET* fusions were associated with older age, origin on the right side, wild-type *RAS* and *BRAF*, MSI-high tumors, as well as shorter survival [12]. In this study, *RET* mutations were enriched in younger and male patients. We did not observe significant correlations between the presence of *RET* mutations on one hand and primary tumor location and *RAS* or *BRAF* status on the other hand. These results suggest that factors affecting the incidence of *RET* mutations are different from those associated with *RET* rearrangements.

Our study had few limitations, such as a limited sample size and retrospective bias. The identified *RET* mutations in this study could be driver aberrations or only neutral passengers. We could not distinguish

between them owing to limited information regarding *RET* mutations in mCRC. Furthermore, because the follow-up time was limited, overall survival could not be determined.

Conclusions

Despite these limitations, our study suggests that tumors with *RET* mutation represent a novel subtype of mCRC. Further studies are needed to evaluate the efficacy of RET inhibitors in patients with RET-mutated mCRC.

Abbreviations

RET
rearranged during transfection
MTC
medullary thyroid cancer
NSCLC
non-small cell lung cancer
CRC
colorectal cancer
mCRC
metastatic colorectal cancer
MSI
microsatellite instability
TMB
tumor mutation burden
PFS
progression-free survival

Declarations

Ethics approval and consent to participate:

The study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center (GZKJ2020-023).

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets analyzed during the current study are available in the Research Data Deposit system of Sun Yat-sen University Cancer.

Competing interests:

The authors declare that they have no competing interests

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Authors' contributions

Concept and design: All authors.

Acquisition, analysis, or interpretation of data: Yuan-Zhong Yang and Wan-Ming Hu.

Drafting of the manuscript: Wen-Zhuo He and Liang-Ping Xia.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Wen-Zhuo He, Wan-Ming Hu and Liang-Ping Xia.

Obtained funding: Wen-Zhuo He.

Administrative, technical, or material support: Yuan-Zhong Yang and Wan-Ming Hu.

Supervision: Liang-Ping Xia.

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Figures

Figure 1

Flow chart describing patient selection for this study.

Figure 2

Frequent RET mutations detected in 582 patients with metastatic colorectal cancer.

Figure 3

Comparison of progression-free survival between patients with RET wild-type and RET-mutated tumors who were administered oxaliplatin-based first-line chemotherapy.

Supplementary Files

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