Clinicopathological and molecular characteristics of early-onset vs late-onset colorectal cancer according to tumor location

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Abstract
Background The incidence of early-onset colorectal cancer (EOCRC) is rapidly increasing worldwide in decade when screening of colorectal cancer (CRC) is more prevalent. The clinicopathological and molecular characteristics of EOCRC have not yet been clarified. This study aims to evaluate clinicopathological and molecular features among EOCRC and late-onset colorectal cancer (LOCRC) patients according to different tumor locations.
Methods We identified CRC patients from a prospectively maintained CRC database between January 2015 and December 2018. The clinicopathological and molecular characteristics including dMMR, mutation of PIK3CA, BRAF and KRAS were compared between EOCRC and LOCRC. The relationships according to different tumor locations were assessed.
Results Totally 4468 patients were analyzed in this study. Compared to LOCRC patients, EOCRC patients were more likely to have status of dMMR (OR, 2.52; P < 0.001), regardless of tumor location. EOCRC patients were more likely to be detected with mutation of PIK3CA (OR, 1.24; P = 0.041), which only tended to exist in the left-side colon (OR, 1.51; P = 0.06), but not in the right-side colon or rectum. No significant difference was found for BRAF or KRAS mutation, but mutation of KRAS was more frequently found in left-side colon (OR, 1.34; P = 0.04) among EOCRC patients.
Conclusion Status of dMMR, mutation of PIK3CA, BRAF and KRAS was different between EOCRC and LOCRC patients according to different tumor locations, which implied that EOCRC might be a unique subgroup of CRC patients. Further investigations of molecular and genetic differences should be performed to help define new diagnosing and therapeutical strategies for EOCRC patients.

Keywords Early-onset colorectal cancer · Molecular characteristics · Mismatch repair deficiency · Tumor location

Introduction
Colorectal cancer (CRC) is the third most common and the second most lethal cancer worldwide [1], which has been found to have a higher incidence with increasing age. Due to benefits of CRC screening, CRC incidence has declined overall or been stable in developed countries in the last decades. In contrast, the incidence of CRC among individuals younger than 50 years of age (early-onset CRC, EOCRC) is on a significant rise [2, 3]. It is estimated that around 11% of colon cancers and 18% of rectal cancers occur in individuals younger than 50 years of age [4]. Previous studies suggested that EOCRC patients might be a specific subgroup that tended to have a higher prevalence of low-grade tumor differentiation, to exhibit an advanced stage, and to present with or develop to metastatic disease [5]. However, whether EOCRC patients have a distinct biological behavior remains controversial.
Status of mismatch repair (MMR) genes and mutation of PIK3CA, BRAF and KRAS were important biomarkers to evaluate CRC. Microsatellite instability (MSI) tumors, whose carcinogenic pathway is also known as the “Mutator Phenotype pathway”, represent 10–15% of all CRCs [6]. The underlying pathogenesis may relate to presence of germ-line mutations in MMR genes, which results in MSI or deficiencies of mismatch repair genes (dMMR) [7]. dMMR could be found in 15% of sporadic CRC, mainly due to an epigenetic inactivation of MLH1 [8]. Palomba G et al. reported an overall mutation rate were 35.6% for KRAS, 2.1% for BRAF, and 14.3% for PIK3CA gene in CRC [9]. A comprehensive genetic characterization of EOCRC in a large cohort is lacking, thus the difference of dMMR status, mutation of KRAS, BRAF, PIK3CA genes between EOCRC and LOCRC patients still needs further research, as well as their difference according to different tumor locations. To fill this knowledge gap, we attempted to investigate the clinicopathological and molecular characteristics between EOCRC and LOCRC patients, and their difference according to different tumor locations, therefore stratifying CRC patients by holding up the age of onset and tumor location.

Methods

Patients’ selection

This study was in accordance with the Helsinki Declaration and approved by the institutional review board of the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Patients diagnosed with CRC who underwent surgery for colorectal adenocarcinoma from January 2015 to December 2018 were identified at the Sixth Affiliated Hospital of Sun Yat-sen University from a prospectively maintained CRC database. Exclusion criteria were: (1) patients younger than 18 or older than 90 years; (2) recurrent CRC; (3) patients with multiprimary cancer; (4) patients with substantial missing data. EOCRC patients were defined as those diagnosed with CRC under age of 50 years old in this study. Data of clinicopathological and molecular variables were collected including age, gender, body mass index, and information of tumor (location, stage, differentiation and molecular testing). After manual review, there were total 4468 patients included in this study (947 EOCRC cases and 3521 LOCRC cases). Flow chart of patient selection was shown in Fig. 1.

Definition of variables

Tumor staging was performed according to the 8th Edition of American Joint Committee on Cancer TNM staging system. Tumor differentiation was categorized as well differentiated, moderately differentiated, poorly differentiated, undifferentiated carcinoma, mucinous carcinoma, or signet-ring cell tumor. The primary tumor location site was categorized as right-side colon if the tumor was located above the splenic flexure (including cecum, ascending colon and transverse colon), and left-side colon if it was located at or below the splenic flexure and above rectum (including descending colon and sigmoid colon), and rectum.

Fig. 1 Flow chart of patient selection

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Status of MMR were examined using immunohistochemical (IHC) staining for MLH1, MSH2, MSH6, and PMS2 protein. Briefly, formalin-fixed paraffin-embedded tissues were serially cut into 4 μm sections on silanized glass slides. Slides were deparaffinized with dimethylbenzene and rehydrated through graded alcohols before retrieving antigen by incubation in sodium citrate buffer. Endogenous peroxidase was blocked with hydrogen peroxide solution and then incubated with anti MLH1, MSH2, MSH6, and PMS2 at 4 °C overnight. Slides were stained with diaminobenzidine in an Envision System and counterstained hematoxylin. Cases with complete nuclear loss of MMR expression in invasive tumor cells but with retained expression in inflammatory cells and/or adjacent normal tissue as positive controls were considered MMR deficiency.

Mutation of PIK3CA, BRAF and KRAS genes were examined using polymerase chain reaction (PCR), including mutation of exon9 and exon20 of PIK3CA, BRAF V600E and exon2, exon3 and exon4 of KRAS. Sequences were listed in Table 1.

### Table 1 Sequence for PIK3CA, BRAF and KRAS genes

| Gene | Exon | Forward primer | Reverse primer |
|------|------|----------------|----------------|
| PIK3CA | 9 | ATCTGGTCTTGTATGCTGCTT | GGTATGGTAAAAACATGCTGAGA |
| PIK3CA | 20 | CATTGCTCAAACACTGACCA | TCTCTATGCAATCGGTCTTTT |
| BRAF V600E | | ATCCACCTCCTAATAATCAAGA | TAAACCTTTTGAAAGGGGC |
| KRAS | 2 | TCAAGTCCITTGGCCCATTTT | TGCATGGCATTAGCAAAAGAC |
| KRAS | 3 | TTGTGGACAGGTITTTGAAAGA | AGAAGCAATGCCCTCCTCAAG |
| KRAS | 4 | GTGTGACATGGTCTATATAGTC | GAATGGTCTCTGACCAGTAA |

Results

### Clinicopathological characteristics

A total of 4468 patients with CRC were included in this study, among whom 947 patients (21.2%) were EOCRC and 3521 patients (78.8%) were LOCRC. 2730 (61.1%) patients were male and 1738 (39.1%) were female, with a gender ratio of 1.53. Patients with EOCRC were significantly likely to have family history (P<0.001), while patients with LOCRC tended to with early stage (stage I or II, P=0.010). About one half (47.9%) of tumors were located in rectum. Data of clinicopathological characteristics of patients were shown in Table 2.
Survival analysis

The median follow-up time was 31.82 (interquartile range 22.91–45.75) months. The 3-year overall survival (OS) rate of EOCRC was 82.58%, which was significantly higher than LOCRC (76.98%, P = 0.001). However, the 3-year disease free survival (DFS) rate was significantly worse in EOCRC than LOCRC (70.90% Vs. 75.05%, P = 0.028). Survival curves were shown in Fig. 2.

Status of dMMR, mutation of PIK3CA, BRAF and KRAS

Overall, 10.2% patients had status of dMMR; while 12.2%, 2.84% and 45.7% patients were detected with PIK3CA mutation, BRAF mutation and KRAS mutation, respectively. Compared with LOCRC patients, EOCRC patients were more likely to have dMMR status (18.1% vs. 8.04%, odds ratio [OR], 2.52; confidence interval [CI], 2.05–3.10; P < 0.001). Negative status of MLH1 (OR, 2.11; CI, 1.55–2.87; P < 0.001), MSH2 (OR, 4.31; CI, 2.86–6.48; P < 0.001), MSH6 (OR, 3.40; CI, 2.42–4.76; P < 0.001), PMS2 (OR, 1.83; CI, 1.42–2.35; P < 0.001) were all observed more frequently in patients with EOCRC. EOCRC patients were more likely to detected with mutation of PIK3CA (14.1% vs. 11.7%, OR, 1.24; CI, 1.01–1.53; P = 0.041), especially at exon20 (OR, 1.88; CI, 1.36–2.60; P < 0.001). No significant difference was found for BRAF or KRAS mutation (Table 3).

Status of dMMR, mutation of PIK3CA, BRAF and KRAS in different tumor locations

Compared with LOCRC patients, status of dMMR was detected more frequently in EOCRC, regardless of location of tumor, so were loss of MSH2 and MSH6. Loss of MLH1 and PMS2 were detected more frequently in EOCRC in colon including both left side (OR, 3.64; CI 1.85–7.16; P < 0.001; and OR, 2.50; CI 1.44–4.35; P = 0.001, respectively) and right side (OR, 1.85; CI 1.23–2.78; P = 0.003; and OR, 1.97; CI 1.38–2.82; P < 0.001, respectively), but not in rectum.

As mentioned above, EOCRC patients were more likely to detected with mutation of PIK3CA, which only trended to exist in left-sided colon (OR, 1.51; CI 0.98–2.33; P = 0.06), but not in right-sided colon or rectum. Overall, no significant difference was found for BRAF or KRAS mutation between EOCRC and LOCRC, but mutation of KRAS was more frequently in EOCRC than LOCRC in left-sided colon (OR, 1.34; CI 1.02–1.77; P = 0.04), especially mutation at exon 2 (OR, 1.60; CI 1.21–2.12; P = 0.001), but not in right-sided colon or rectum (Table 4).

Discussion

Mounting evidence indicates that EOCRC has unique molecular profiles, which might influence disease outcomes and response to therapy [10]. With regard to MMR status, recent studies found that dMMR was more prevalent in EOCRC.
patients. Pan Li et al. [11] reported a higher incidence of dMMR in younger group of patients (13.8%) versus the middle-aged (12.2%) or older-aged patients (7.9%) \((P < 0.001)\), and young CRC patients with dMMR had higher OS than young patients with pMMR \((P = 0.03)\). R Gryfe et al. [12] also reported a high-frequency of dMMR in 17% of colorectal cancers in EOCRC patients. Likewise, in this study, we found that about 10.2% patients with CRC had status of dMMR on the whole, and it was detected more frequently in EOCRC patients (18.1%) versus LOCRC patients (8.0%) \((P < 0.001)\). What’s more, in this study, we found that dMMR rate was significant different according to tumor location between EOCRC and LOCRC patients. Compared with LOCRC patients, EOCRC patients were more likely to have status of dMMR regardless of location of tumor, so were loss of MSH2 and MSH6. Loss of MLH1 and PMS2 were detected more frequently in EOCRC overall, but only in colon including both left side and right side rather than in rectum.

Mutation of \(PIK3CA\), \(BRAF\) and \(KRAS\) is mainly focus on CRC patients with recurrence or metastasis. They are important potential biomarkers for prognosis as well as targeted therapies [13, 14]. Though their influence on therapeutic strategy in non-recurrent or non-metastatic CRC was unknown, frequency of such mutation and the association with clinicopathological variables were more and more reported in recent years [15–17].

In a meta-analysis of \(PIK3CA\) by Shuofei Yang, et al. forty-four studies enrolling 17,621 patients were eligible and the rate of \(PIK3CA\) mutation was 12.9% and was associated with proximal tumor location [18]. In the present study, mutations of \(PIK3CA\) was detected in 12.2% patients overall, and more likely in EOCRC than LOCRC, but not associated with tumor location significantly.

As previously reported, mutation of \(KRAS\) is an early event in the carcinogenesis of CRC and its incidence in CRC patients is about 30% to 50% [19]. In our study, the incidence of \(KRAS\) mutation was 46.8%, which was similar to those in studies of Eastern and Western populations [15]. Most previous studies reported a higher incidence of \(KRAS\) mutations in EOCRC patients, which may due to \(KRAS\) mutations usually associate with more advanced tumor stage in CRC patients [20]. Pilozzi E et al. [19] reported that the EOCRC with left-sided tumors showed \(KRAS\) mutation in 43% of cases; in contrast, in the LOCRC, \(KRAS\) mutation was observed in 14% of cases. Gunal et al. [20] showed a very high incidence of \(KRAS\) mutations in patients younger than 40 years compared with those older than 40 years (66.7% and 36%, respectively). In our study, there was no significant difference of \(KRAS\) mutations incidence between EOCRC and LOCRC patients, but mutation of \(KRAS\) was more frequently in EOCRC than LOCRC in left-sided colon \((OR, 1.34; CI 1.02–1.77; P = 0.04)\), but not in right-sided colon or rectum.

Frequency of \(BRAF\) mutation was 10.8% in meta-analysis with about 12 thousand patients, and was found associated with advanced TNM stage, poor differentiation [21]. In our study, we didn’t find a significant difference for \(BRAF\) mutation in age and tumor location.

### Table 3  Comparisons on status of dMMR, mutation of \(PIK3CA\), \(BRAF\) and \(KRAS\) between EOCRC and LOCRC patients

| Abbreviation | Overall \((n = 4468)\) | EOCRC \((n = 947)\) | LOCRC \((n = 3521)\) | OR (95% CI) | \(P\) value |
|-------------|-----------------|-----------------|-----------------|-------------|----------|
| dMMR        | 454 (10.2%)     | 171 (18.1%)     | 283 (8.04%)     | 2.52 (2.05, 3.10) | \(< 0.001^*\) |
| MLH1 negative | 187 (4.19%)     | 66 (6.97%)      | 121 (3.44%)     | 2.11 (1.55, 2.87) | \(< 0.001^*\) |
| MSH2 negative | 95 (2.13)       | 50 (5.28%)      | 45 (1.28%)      | 4.31 (2.86, 6.48) | \(< 0.001^*\) |
| MSH6 negative | 142 (3.18)      | 66 (6.97%)      | 76 (2.16%)      | 3.40 (2.42, 4.76) | \(< 0.001^*\) |
| PMS2 negative | 304 (6.80)      | 97 (10.2%)      | 207 (5.88%)     | 1.83 (1.42, 2.35) | \(< 0.001^*\) |
| \(PIK3CA\) mutation | 546 (12.2%) | 134 (14.1%) | 412 (11.7%) | 1.24 (1.01, 1.53) | 0.041* |
| exon9 mutation | 376 (8.42%) | 77 (8.13%) | 299 (8.49%) | 0.95 (0.73, 1.24) | 0.722 |
| exon20 mutation | 173 (3.87%) | 57 (6.02%) | 116 (3.29%) | 1.88 (1.36, 2.60) | \(< 0.001^*\) |
| \(BRAF\) mutation | 127 (2.84%) | 28 (2.96%) | 99 (2.81%) | 1.05 (0.69, 1.61) | 0.812 |
| \(KRAS\) mutation | 2041 (45.7%) | 427 (45.1%) | 1614 (45.8%) | 0.97 (0.84, 1.12) | 0.768 |
| exon2 mutation | 1743 (39.0%) | 362 (38.2%) | 1381 (39.2%) | 0.96 (0.83, 1.11) | 0.577 |
| exon3 mutation | 103 (2.31%) | 20 (2.11%) | 83 (2.36%) | 0.89 (0.55, 1.46) | 0.654 |
| exon4 mutation | 202 (4.52%) | 48 (5.07%) | 154 (4.37%) | 1.17 (0.84, 1.63) | 0.363 |

Abbreviation: EOCRC early-onset colorectal cancer, LOCRC late-onset colorectal cancer, OR odds ration, CI confidence interval. *\(P\) value with statistic significance.
### Table 4 Comparisons on status of dMMR, mutation of PIK3CA, BRAF and KRAS between EOCRC and LOCRC patients according to different tumor locations

|                | Rectum (n = 2138) | Left-sided colon (n = 1267) | Right-sided colon (n = 1063) |
|----------------|-------------------|----------------------------|----------------------------|
|                | ≥ 50 (n = 1712)   | < 50 (n = 426)             | ≥ 50 (n = 813)              | < 50 (n = 250)            |
| **dMMR**       |                   |                            |                            |                            |
| MLH1 negative  | 25                | 8                          | 18                         | 78                        | 1.83 (1.23, 2.72) | 0.003* |
| MSH2 negative  | 7                 | 13                         | 5                          | 14                        | 0.53 | <0.001* |
| MSH6 negative  | 13                | 19                         | 5                          | 12                        | 7.67 (3.04, 19.3) | <0.001* |
| PMS2 negative  | 65                | 17                         | 34                         | 22                        | 1.05 (0.61, 1.82) | 0.05* |
| PIK3CA mutation| 156               | 41                         | 81                         | 32                        | 1.06 (0.74, 1.53) | 0.74 |
| exon9 mutation | 114               | 23                         | 60                         | 25                        | 0.80 (0.50, 1.27) | 0.34 |
| exon20 mutation| 43                | 18                         | 21                         | 7                         | 1.71 (0.98, 3.00) | 0.06 |
| BRAF mutation  | 17                | 7                          | 16                         | 4                         | 1.67 (0.69, 4.04) | 0.26 |
| KRAS mutation  | 822               | 185                        | 343                        | 112                       | 0.83 (0.67, 1.03) | 0.09 |
| exon2 mutation | 709               | 155                        | 282                        | 105                       | 0.81 (0.65, 1.01) | 0.06 |
| exon3 mutation | 32                | 10                         | 53                         | 28                        | 1.26 (0.62, 2.59) | 0.53 |
| exon4 mutation | 84                | 20                         | 85                         | 33                        | 0.95 (0.58, 1.57) | 0.85 |

**Abbreviation:** EOCRC early-onset colorectal cancer, LOCRC late-onset colorectal cancer, OR odds ratio, CI confidence interval. *P* value with statistic significance.
Conclusion

In this retrospective study of patients with EOCRC vs LOCRC, we found status of dMMR, mutation of PIK3CA and KRAS were different between EOCRC and LOCRC patients according to different tumor locations, which implied that EOCRC may be a unique subgroup of CRC patients. Further investigations of molecular and genetic differences should be performed to help define new diagnosing and therapeutical strategies for EOCRC patients.

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Declarations

Conflict of interests The authors declare that they have no competing interests.

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