



Differences Regarding the Molecular Features and Gut Microbiota Between Right and Left Colon Cancer

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For many years, developmental and physiological differences have been known to exist between anatomic segments of the colorectum. Because of different outcomes, prognoses, and clinical responses to chemotherapy, the distinction between right colon cancer (RCC) and left colon cancer (LCC) has gained attention. Furthermore, variations in the molecular features and gut microbiota between right and LCCs have recently been a hot research topic. CpG island methylator phenotype-high, microsatellite instability-high colorectal cancers are more likely to occur on the right side whereas tumors with chromosomal instability have been detected in approximately 75% of LCC patients and 30% of RCC patients. The mutation rates of oncogenes and tumor suppressor genes also differ between RCC and LCC patients. Biofilm is more abundant in RCC patients than LCC patients, as are *Prevotella*, *Selenomonas*, and *Peptostreptococcus*. Conversely, *Fusobacterium*, *Escherichia/Shigella*, and *Leptotrichia* are more abundant in LCC patients compared to RCC patients. Distinctive characteristics are apparent in terms of molecular features and gut microbiota between right and LCC. However, how or to what extent these differences influence diverging oncologic outcomes remains unclear. Further clinical and translational studies are needed to elucidate the causative relationship between primary tumor location and prognosis.

Keywords: Colonic neoplasms; Molecular subtype; Gastrointestinal microbiome; Treatment outcome

INTRODUCTION

Colorectal cancer (CRC) can be characterized by the location of the primary tumor in the colorectum. For many years, developmental and physiological differences have been known to exist between anatomic segments of the colorectum, and CRCs have been known to occur with distinctly different frequencies at different subsites [1]. The proximal and the distal colon have different embryologic origins. The distal duodenum to the proximal

two-thirds of the transverse colon is derived from the midgut whereas the distal third of the transverse colon to the upper two-thirds of the anorectal canal is derived from the hindgut [2]. In addition, they have different physiological functions: the water and electrolyte absorption capacity of the distal colon differs from that of the proximal colon. The main location for water absorption is the proximal colon whereas the main function of the distal colon is the passage of bowel contents.

Recently, the different outcomes, prognoses, and clinical responses to chemotherapy observed between right colon cancer (RCC) and left colon cancer (LCC) have attracted attention. Some trials in terms of metastatic CRC showed that outcomes for patients with left-sided tumors were superior to those for patients with right-sided tumors [3, 4]. With regard to patients who received a curative resection for nonmetastatic colon cancer, the prognostic role of the primary tumor's location is still being debated [5]. In fact, several recent studies have indicated that the sidedness of the primary tumor may be prognostic and predictive of the response to anti-epidermal growth factor receptor (EGFR)

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therapy in metastatic CRC. Trials using cetuximab as an anti-EGFR therapy, including CRYSTAL and FIRE-3, showed that the outcomes for patients with left-sided tumors were superior to those for patients with right-sided tumors [4].

The aim of this review is to describe the differences regarding the molecular features and gut microbiota between right and LCCs based on current evidence. This review article is exempt from the requirement for approval by the Ethics Committee.

DIFFERENT MOLECULAR FEATURES

CRCs exhibit variable genetic signatures and develop through at least three major pathways: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) (Fig. 1).

Chromosomal instability

Of the three major pathways leading to CRC, CIN was the first pathway to be described and is the most commonly seen in 65%–70% of sporadic CRCs [6-8]. Although CIN is one of the most described pathways, its mechanism is still not clear. Several mechanisms leading to CIN include chromosome segregation defects (either defects in mitotic arrest deficient and budding uninhibited by benzimidazoles genes, which are mitotic checkpoints, or abnormal centrosome function or number), telomere dysfunction (either shortened telomeres, often seen in early carcinogenesis, or elongated telomeres due to increased telomerase activity, often seen in advanced stages of CRC), errors in DNA damage repair response affecting *TP53* and *APC* genes, and lastly, loss of heterozygosity (either from mitotic nondisjunction, recombination of chromosomes, or chromosome deletion) [7]. In karyotypic studies, CIN shows a gain or loss of chromosomes. Furthermore, these chromosomes show losses in heterozygosity, i.e., loss of a maternal or paternal allele and gain of the opposite (often abnormal) allele [9]. Loss of heterozygosity is a hallmark feature in CIN-positive tumors, with at least 25%–30% alleles being lost in these tumors [7]. CIN-positive tumors also feature extensive so-

matic copy number alterations (SCNA) in the genome, giving rise to aneuploid tumors from asymmetric mitosis [8].

Microsatellite instability

A microsatellite is defined as a part of DNA that repeats 1 to 6 short nucleotide sequences. MSI is a genetic instability in short nucleotide repeats (microsatellite) due to a high mutation rate as a result of abnormal DNA mismatch repair. The National Cancer Institute suggested panel markers, such as mononucleotide marker (BAT26, BAT25) and dinucleotide marker (D5S346, D2S123, and D17S250), in CRCs. Tumors with MSI show instability in 2 or more markers whilst tumors with microsatellite stability (MSS) show instability in no more than one marker [10]. According to a Bethesda guideline, MSI-High is defined as having instability of 40% or more, MSI-Low as less than 40%, and MSS as less instability. However, in general, MSI-L (instability <40% of markers) CRCs are classified in the same subtype as MSS CRCs. MSI is a characteristic seen in patients with hereditary nonpolyposis colorectal cancer (HNPCC). HNPCC, also called Lynch syndrome, occurs in about 1% to 6% of all CRCs. Patients with Lynch syndrome have an increased risk for a number of extracolonic cancers, including carcinomas of the endometrium, ovary, renal pelvis and ureter, small intestine, stomach, and hepatobiliary tract. HNPCC is characterized by extensive MSI-H, which is due to germ-line mutation of mismatch repair genes [11]. MSI is reported in about 10%–15% of sporadic CRCs. MSI in sporadic CRCs is mainly due to transcriptional silencing by acquired promoter hypermethylation of the *hMLH1* gene [12].

In general, MSI CRCs are clinically characterized by poorly differentiated tumors in the proximal colon of older females that exhibit mucinous or signet-ring cell histology. MSI CRCs are clinically characterized as having a favorable prognosis. In addition, MSI is a potential sensitive marker for 5-fluorouracil (5-FU) therapy. Recent studies suggest that MSI is a marker of good response to 5-FU treatment, particularly when accompanied by large deletions in *HSPH1* (*HSP110*) [13, 14].

CpG island methylator phenotype

Epigenetic changes are physiological mechanisms that regulate gene expression without altering the DNA sequence. An example of an epigenetic change is methylation of gene promoters, as seen in methylation in a CpG dinucleotide context [15]. CIMP changes in CRC are often defined as excessive methylation of genetic loci that contain CpG islands, usually a promoter of a tumor-suppressor gene, which leads to inhibition of transcription of that gene and promotion of carcinogenesis [8, 15]. The CIMP definition criteria vary among studies, with some using at least three loci methylated from 5 to 15 marker panels and different cutoff values to group them as either CIMP-positive (which is further grouped as CIMP-high and CIMP-low) or CIMP-negative [8, 16]. The CIMP definition criteria and CRC prognosis due to high heterogeneity in the CIMP definitions are still serious issues; however, a



Fig. 1. Molecular features of right and left colon cancers. AREG, amphiregulin; CIMP, CpG island methylator phenotype; SCNA, somatic copy number alterations; EGFR, epidermal growth factor receptor; EREG, epiregulin; MSI, microsatellite instability; VEGF-1, vascular endothelial growth factor 1.

CIMP-positive or a CIMP-high CRC may be an independent prognostic factor for poor survival compared to CIMP-negative CRC [16]. CRCs diagnosed within five years postcolonoscopy are usually CIMP-positive [17]. The association of CIMP with MSI showed poorer survival in patients with CRC [8, 16]. The results of predicting responses to therapies by using CIMP definitions are still inconsistent [16]. CIMP-positivity may be seen in sessile serrated lesions (SSL) in 15%–30% of patients with CRC [15].

RIGHT VERSUS LEFT: DIFFERENT MOLECULAR CHARACTERISTICS

Traditionally, primary tumors arising from the left and the right sides of the colon have distinct chromosomal and molecular characteristics. CIMP-high, MSI-high CRCs are more likely to occur on the right side [18, 19] whereas tumors with CIN have been detected in approximately 75% of patients with LCC and 30% of those with RCC [20]. The mutation rates of oncogenes and tumor suppressor genes also differ between RCC and LCC. Hypermethylation is more common in RCC than LCC [21], and RCCs have also been associated with an increase in RAS and phosphoinositide 3-kinase pathway mutations and a higher frequency of transforming growth factor (TGF)- β 2 mutations and *BRAF* mutations [22]. Mutations in the *APC*, *SMAD4*, and *TP53* genes occur more often in LCC than in RCC [23]. Overexpression of the EGFR ligands epiregulin (EREG) and amphiregulin (AREG) and amplifications of EGFR and human EGFR2 are associated with LCC [18, 21]. High expressions of EREG and AREG in tumors are associated with better response rates and improved outcomes to anti-EGFR antibody therapy in patients with *KRAS* and *NRAS* wild type (wt) metastatic CRCs [24]. The expression of vascular endothelial growth factor 1 is also significantly higher in LCC than in RCC [25]. Therefore, variable treatment options should be provided because mutations and genomic patterns are variable.

CRC gene expression profiling (CMS classification)

Recently, several studies performed gene expression profiling to categorize CRCs into subtypes and identify associations with genes and clinicopathological features. Members of the Colorectal Cancer Subtyping Consortium combined genomic datasets for a total of 4,151 samples to perform consensus molecular subtyping (CMS) by applying unsupervised clustering techniques [26]. Extensive labor established four CMSs. CMS1 (MSI immune, 14%) is characterized as presenting with MSI and an activated immune system; the tumors are CIMP-positive and SCNA-low, harbor *BRAF* mutations, and occur in the proximal colon of older female patients. CMS2 (canonical, 37%) is characterized as showing MSS, CIN, and WNT/MYC pathway activation; the tumors are CIMP-negative and SCNA-high with *APC* and *TP53* mutations and occur in the distal colon to rectum. This subtype shows good survival after relapse. CMS3 (metabolic, 13%) is characterized as showing MSS, having a CIMP-low and SCNA-intermediate phe-

notype, showing *KRAS* and *APC* mutations, and exhibiting an epithelial signature and metabolic dysregulation. CMS4 (mesenchymal, 23%) is characterized as showing MSS, having a CIMP-negative and SCNA-high phenotype and occurring at advanced stages. This subtype shows poorer overall survival and signatures of TGF- β activation, stromal infiltration, epithelial-mesenchymal transition activation, matrix remodeling, and angiogenesis. Although, this CMS classification system was not a therapeutic aim, it facilitated a better understanding of the broad biological groups in the overall category of CRCs.

CRC subtype classification using key molecular features

The categorization of CRCs using multiple key molecular features might provide insights regarding various clinical outcomes, although the classification of CRCs is complex because CRCs are heterogeneous. Sinicrope et al. [27] categorized colon cancers into five subtypes with distinct clinicopathological features, including clinical outcomes. This categorization combined *KRAS* and *BRAF*^{V600E} mutations with DNA MMR (mismatch repair) status as key molecular features. In addition, it used a cohort of patients with stage III colon cancer in an adjuvant chemotherapy trial. MMR-proficient tumors with *BRAF* or *KRAS* mutations (42% of all cases) showed higher mortality rates than those without this phenotype. MMR-proficient tumors with *BRAF* wt and *KRAS* wt (49%) were the most prevalent subtype in the cohort and were associated with better survival than tumors lacking this phenotype [27, 28].

Phipps et al. [29] suggested that the combination of MSI and CIMP status and *BRAF* and *KRAS* mutations divided CRCs into 5 categories with distinct clinicopathological features. Type 1 CRCs (7% of all cases) were characterized as having MSI and *BRAF* mutations, were *KRAS* wt- and CIMP-positive, and occurred in the proximal colon of older female patients. Type 2 CRCs (4%) had the highest mortality rate and were defined as having MSS and *BRAF* mutations, as well as being *KRAS* wt- and CIMP-positive. Type 4 CRCs (47%), defined as being MSS-, *BRAF* wt-, *KRAS* wt-, and CIMP-negative, represented the most common subtype, were characterized by canonical *APC* mutations and occurred in the distal colon to rectum of male patients. Type 5 CRCs (7%), defined as being MSI-, *BRAF* wt-, *KRAS* wt- and CIMP-negative, showed the lowest mortality rates and were characterized clinically based on occurrence in the proximal colon of relatively young patients.

Another study reported that CIMP might be used as a molecular marker to determine the poor prognosis of CRC patients with MSS and *BRAF* mutations [30]. This corresponds to type 2 CRCs in the study by Phipps et al. [29].

DIFFERENCES IN GUT MICROBIOTA

CRCs have multiple causes, one of which is the gut microbiome. One study [31] suggested that the gut microbiome may influence

Table 1. Different clinical features and gut microbiota between right and left colon cancers

Feature	Right	Left
Age at diagnosis	Older	Younger
Sex	More women	More men
Tumor size	Larger	Smaller
Tumor condition at diagnosis	More advanced	Less advanced
Differentiation	Poor	Well
Prognosis	Poor	Good
Biofilm	Abundant	Less abundant
Microbiome	<i>Prevotella</i>	<i>Fusobacterium</i>
	-	<i>Escherichia/Shigella</i>
	<i>Selenomonas</i>	<i>Leptotrichia</i>
	<i>Peptostreptococcus</i>	-
Bile salt	Abundant	Less abundant
Association with Western dietary pattern	Less	More

not only the initiating events of carcinogenesis but also its progression (Table 1).

How bacteria influence CRC initiation

Two major theories have emerged for how bacteria might initiate CRCs [32, 33]. The first and perhaps most direct is that certain bacteria have DNA mutagenesis capabilities and/or interfere with host DNA repair machinery, which has been observed in enterotoxigenic *Bacteroides fragilis* that express *B. fragilis* toxin (BFT), superoxide-producing *E. faecalis*, and the polyketide synthase (*pks*)-expressing clade of *Escherichia coli*. The second theory considers that many implicated bacteria, including the above three species, as well as *Fusobacterium nucleatum*, share the ability to enhance Wnt-mediated signaling pathways or other proinflammatory pathways that are commonly mutated and/or overexpressed in CRC. However, that a single organism is responsible for all CRCs is highly unlikely.

An emerging concept in the role of microbiota in CRC initiation is that both the composition of the microbiota and the complex community structures they form, such as bacterial biofilms, also dramatically alter both host and microbial functions in CRCs. Bacterial biofilms along the colorectal axis are present in approximately 15% of healthy patients upon colonoscopy [34], but were recently shown to be a feature in nearly 100% of patients with right-sided CRC [35]. However, the reason bacteria preferentially form biofilms on RCC is still not fully understood. In healthy individuals, approximately 15% exhibit thin biofilms, although they are not specific to the proximal colon [35]. Thus, other environmental influences, such as diet and smoking, may affect biofilm development [36].

Biofilms are defined as massive bacterial invasions of the mucus layer that are encased in a polymeric matrix. However, why or

how microbiota form biofilms in the colon is still not clear. One hypothesis is that biofilm formation is a microbiota defense mechanism against the host [37]. Approximately 100 species of bacteria can exist as biofilms; the predominant species is bacteroidetes (encompassing *Bacteroides* and *Prevotella*). Biofilms in CRC patients tend to be thicker and more continuous than those in healthy controls [35, 38]. Tissues underlying the biofilms in CRC patients showed decreased or altered E-cadherin and enhanced interleukin-6 and Ki67 expression in the tumor host, as well as phospho-Stat3, suggesting that the biofilms elicited a procarcinogenic effect [35]. This finding is perhaps unsurprising given that a feature of biofilms is the invasion of the mucus layer by bacteria, allowing bacteria to directly interact with colonocytes and potentially trigger inflammatory responses, as well as oncogenic changes, in the colonic epithelial cell layer. Conversely, colonic epithelial cells and/or leukocytes have been observed invading the biofilms, again suggesting that the biofilms are immunogenic and involve highly dynamic bacteria-host interactions [35].

How bacteria influence CRC progression

Observational studies on patient outcomes have provided clues as to which microbes are associated with CRC progression. Boleij et al. [39] showed that BFT was more often observed in advanced CRC cases than in early-stage CRCs. Basically, some studies suggested that *Fusobacterium* was abundant in right-side colon cancer and might be linked to the worse prognosis [40, 41]. Another study by Castellarin et al. [42] demonstrated that a high level of *F. nucleatum* DNA in tumor tissue was associated with an increased number of lymph node metastases. In addition, Mima et al. [43] showed that *F. nucleatum* was associated with a decrease in CD3+ T cells within CRC tumors, a feature that is typically associated with MSS status and poorer patient outcomes [44].

Prevotella, *Selenomonas*, and *Peptostreptococcus* were identified in relatively higher abundances in RCC than in LCC. Conversely, *Fusobacterium*, *Escherichia/Shigella*, and *Leptotrichia* were relatively abundant in LCC compared to RCC [45]. A recent study showed that these CRC-associated microbiota profiles were linked to distinct mucosal gene expression profiles [31]. Furthermore, analysis of CRC microbiomes and their relation to tumor CMSs showed enriched levels of *Fusobacteria* and *Bacteroidetes* and decreased levels of *Firmicutes* and *Proteobacteria* in CMS1. CMS2 was enriched for *Selenomonas* and *Prevotella* species whereas CMS3 showed few significant associations [46]. In addition, a prospective cohort study found that prudent diets rich in whole grains and dietary fiber were associated with a lower risk of *F. nucleatum*-negative cancer, supporting a potential role for intestinal microbiota in mediating the association between diet and CRC [47].

ONCOLOGIC OUTCOMES

Growing evidence indicates that primary tumor location and tu-

mor stage are prognostic factors for patients with CRCs. However, the difference in outcome is related to not only the location of the primary tumor but also its molecular profile. Recently, in *K-RAS* wt metastatic CRC patients receiving anti-EGFR therapy, the molecular characteristics considered typical of RCC more frequently overlapped with CMS1 (MSI immune) whereas CMS3 and CMS4 were recurrent in LCC [18]. The study also showed a correlation between the different molecular characteristics investigated and survival, confirming a consistent link between molecular features and clinical outcome.

CONCLUSIONS

Distinctive aspects regarding the molecular features and gut microbiota exist between RCCs and LCCs. However, how and the extent to which these differences influence divergent oncologic outcomes is still unclear. Thus, further clinical and translational studies are needed to elucidate the causative relationship between primary tumor location and prognosis.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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