Chapter 5
Colorectal Cancers Developed from Proximal and Distal Tumor Location Belong to the Distinct Genetic Entity and Show Different Oncologic Behavior

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Abstract Colorectal cancer is now understood as a genetic disease.

Because of the importance of this highly prevalent disease, intense research efforts during the past two decades have focused on molecular processes to gain a better understanding of carcinogenesis. Since then, colorectal cancer has become a leading research model for the genetic basis of cancer. Attempt of molecular classification of colorectal cancer was made in order to offer precision medicine.

Colorectal cancer located either proximal or distal to the splenic flexure has been considered as belonging to different clinicopathological or physiological categories. Now, tumor location in colorectum is becoming an important surrogate marker to estimate prognosis and to determine the treatment decision including selection of chemotherapy agents for CRC.

Keywords Colorectal cancer • Tumor location • Chemotherapy • Carcinogenesis • Molecular classification

5.1 Introduction

Colorectal cancer (CRC) is the third most common cancers in developed countries [1]. CRC is a significant cause of morbidity and mortality in Western population. Majority of CRC develops from distal part of the colon (descending, sigmoid colon and rectum), but recently the number of CRC develops from proximal part of the colon (cecum, ascending and transverse colon) is gradually increasing especially in elderly female population. Interestingly, majority of CRCs of Lynch syndrome, one of the common

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Y. Shimada, K. Yanaga (eds.), *Molecular Diagnosis and Targeting for Thoracic and Gastrointestinal Malignancy*, Current Human Cell Research and Applications, https://doi.org/10.1007/978-981-10-6469-2_5

hereditary CRC syndromes, develop from proximal part of the colon. The idea that CRCs located either proximal or distal to the splenic flexure of the colon belong to the different clinicopathological or physiological categories was not new. Nearly three decades ago, Bufill proposed that colon cancer located to the proximal and distal part of colorectum may rise from different biological pathways [2]. Differences in the embryologic origin of epithelium of proximal and distal segments may determine the differences in the susceptibility to the environmental carcinogenesis.

Recent advancement in the molecular biology supports an idea that differently accumulated genetic alterations on each side of the colon may underlie the pathologically different colorectal cancer. More recently, primary tumor location of CRCs has been considered as a prognostic factor: patients with proximal-sided tumors have worse prognosis than those with distal-sided tumors. Treatment effect of certain anticancer medicine may be different depending on the tumor location.

In this chapter, tumor location and CRCs are widely discussed especially hereditary, and acquired alterations on proximal or distal part of CRCs are discussed.

5.2 Biology of Normal Colon

Embryologic origins are different between proximal and distal segment of colorectum [3]. Distal part of the colorectum (those originating in the splenic flexure, descending colon, sigmoid colon, rectum) is derived from the embryonic hindgut [3]. In contrast, proximal part of the colorectum (those originated in the cecum, ascending colon, hepatic flexure, or transverse colon) is derived from the embryonic midgut, just as part of the duodenum and small intestine [3]. Vascular supply to the proximal and distal colon is also totally different, as midgut-originated proximal colon is served by the superior mesenteric artery, whereas hindgut-originated distal part of the large intestine is served by the inferior mesenteric artery [3].

Endocrine component is also different in tumor location. Accumulation of chromogranin immunoreactive cells are observed in the distal large intestine and few of those cells are observed in the proximal part of colon [4]. Ornithine decarboxylase (ODC) is a key enzyme in polyamine synthesis, and its functional activity closely parallels cellular proliferative activity in normal colonic mucosa. GTP-activated isoform of ODC is predominantly distributed in proximal colon [5].

5.3 Colorectal Cancer

In general proximal CRCs were more frequently diagnosed in elderly woman, and distal CRCs were more frequently diagnosed in men [6]. Patients with proximal CRC complain less symptoms, and comorbidities were more common in patients with proximal CRC [6]. Histologically, mucinous, undifferentiated, and signet ring cell carcinomas were more frequently diagnosed in patients with proximal CRC.

Consistent with these differences in embryological origin, distal-sided and proximal-sided CRC possess different karyotypic and enzymatic profiles. For example, expression of ODC is frequently elevated in many human neoplasms including CRC. High levels of ODC expression and the presence of a GTP-activated isoform for proximal CRC predict a favorable prognosis in CRC [5]. It is known that cancers developed in the distal part of colon have more unstable in karyotype and show frequent loss of heterozygosity of chrosomose compared to those developed in the proximal colon.

5.3.1 Hereditary Colorectal Cancer

Hereditary CRCs account for approximately 5–10% of the total CRC burden. Genetic germline mutations are the basis of inherited colon cancer syndromes. Two forms of hereditary colorectal cancer syndromes—one with and without associated polyposis of the colon—are known as familial adenomatous polyposis (FAP) and Lynch syndrome (LS). Interestingly CRCs based on FAP often develop in the distal part colon, and those on LS more often develop in proximal part of the colon.

5.3.2 Lynch Syndrome (LS)

LS is the most frequently observed hereditary syndrome developing CRC. It accounts for approximately 3–6% of the total CRC burden. The Lynch syndrome is an autosomal dominant syndrome with 30–74% penetrance. The syndrome is characterized by an onset of CRC at an early age, right-sided predominance, excess of synchronous and metachronous CRCs, and extracolonic tumors of the endometrium, renal pelvis, ureter, and other locations. Pathologic characteristics of CRCs in Lynch syndrome include poor differentiation, mucin production, peritumoral lymphocytic infiltrate, and Crohn's-like reaction. Causing genes for LS are mismatch repair genes including *MLH1*, *MSH2*, *MSH6*, and *PMS2*.

5.3.3 Familial Adenomatous Polyposis (FAP)

FAP is an autosomal-dominant inherited disease characterized by the development of multiple adenomas throughout the colorectum. It represents about 0.5–1% of all CRC cases and is the most common gastrointestinal polyposis syndrome. Germline mutations in the *APC* gene are responsible for most cases of FAP. Classic FAP is characterized by the presence of hundreds to thousands adenomatous polyps throughout the colon and rectum. At the time of adolescence, the polyps are usually identified in the rectosigmoid as small polyps and, thereafter, increase in size and

number. About half of FAP patients develop adenomas by 15 years of age and 95% by age 35 years. CRC inevitably occurs throughout the colorectum at an earlier age than sporadic CRC (average age of 35 years) but mainly occurs in distal part of the colon. Attenuated FAP (AFAP) is a variant of FAP with a mild disease course, characterized by a reduced number of polyps (10–100), later age of onset, frequently proximal-sided distribution of polyps, and lower CRC risk (up to 70%). Clinical definition of AFAP is controversial and should be considered in any patient with 10–99 adenomas, although a precise diagnosis is often difficult in a single patient. In many FAP patients, extracolonic manifestations are present, including gastric and duodenal polyps, desmoid tumors, thyroidal and brain tumors, osteomas, congenital hypertrophy of the retinal pigment epithelium, supernumerary teeth, and epidermoid cysts.

5.4 Molecular Carcinogenesis of Sporadic Colorectal Cancer

Recent advances have contributed to the understanding of the molecular basis of these various patterns of sporadic CRC. CRC develops as a result of the pathologic transformation of normal colonic epithelium to an adenomatous polyp and ultimately an invasive cancer. Mutations in two classes of genes, tumor-suppressor genes and proto-oncogenes, are thought to impact a proliferative advantage to cells and contribute to development of the malignant phenotype. The multistep progression requires years and possibly decades and is accompanied by a number of recently characterized genetic alterations.

Two molecular pathways for colorectal carcinogenesis are well known [7]. Genomic instability is critical for carcinogenesis. It accelerates the neoplastic evolutionary process, and without this, acquisition of new genetic alteration would occur too slowly for cancer development. One common genomic instability is chromosomal instability (CIN) [8]. The molecular model of the adenoma-carcinoma sequence (traditional pathway) is attributed to the CIN, which is characterized by stepwise mutation or deletion of *KRAS*, *APC*, *DCC*, and *TP53* [8, 9] (Fig. 5.1). As a gatekeeper gene, *APC* is an important regulator of the CIN pathway [10]. This pathway is involved in the formation of dysplastic aberrant crypt foci (ACF) with *KRAS* mutations [11]. A minority of dysplastic ACF develops into simple and then advanced adenomatous polyps and finally produces an invasive cancer [12, 13]. Sporadic CRC resulted in CIN pathway mainly develops in distal part of the colon.

The second pathway responsible to the genomic instability is the mutator pathway—microsatellite instability (MIN) pathway. In this pathway, dysfunction of a mismatch repair (MMR) genes (e.g., *MLH1*, *MSH2*, *MSH6*, or *PMS2*) results in genetic instability characterized by the accumulation of numerous mutations specifically target of repetitive DNA sequences called microsatellite. Thus, this phenomenon is termed microsatellite instability (MSI). The high frequency of MSI detected throughout the genome after inactivation of a MMR gene is termed high-level MSI (MSI-H) [14]. A subset (10–15%) of sporadic CRC exhibits MSI-H, and

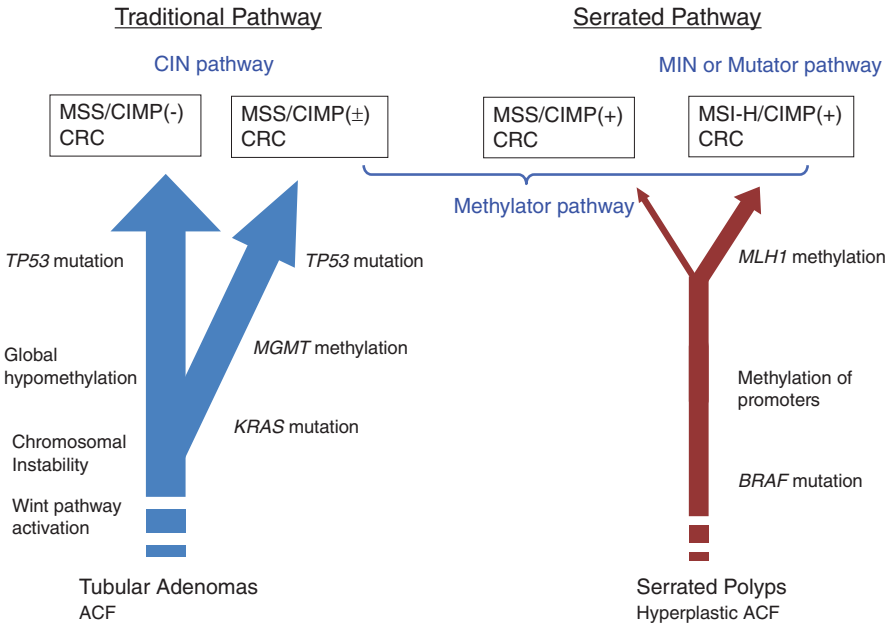

Fig. 5.1 Traditional and serrated pathway of carcinogenesis in colorectal cancer. *CIN* chromosomal instability, *MSS* microsatellite stable, *CIMP* CpG island methylator phenotype, *MIN* microsatellite instability pathway, *MSI*, microsatellite instability, *ACF* aberrant crypt foci (Matsubara [7])

most of those are caused by silencing of *MLH1* due to promoter hypermethylation, one of the epigenetic events that may lead to multiple genetic changes in tumor cells. Sporadic CRC following MIN pathway mainly develops in the proximal portion of the colon.

5.4.1 Methylator Pathway in Colorectal Cancer

Currently a subset of CRC can be distinguished by the status of methylation at several promoter loci. This panel (marker promoters) takes advantage to classify cancers as CpG island methylator phenotype (CIMP+) or not (CIMP-), just like NIH microsatellite panel does to distinguish MSI status [15, 16]. Depending on the marker used, 24–51% of CRCs belong to CIMP+ subtype. The first proposed CIMP panel includes promoter regions of *MLH1*, *p16*, *MINT1*, *2*, and *31* [15]. CIMP+ CRCs are often developed in older women, with a predominance of proximal colon, high grade, and mucinous type. CIMP+ CRCs are associated with hypermethylation of many promoters other than original five markers. Since CIMP CRCs frequently show promoter methylation at *MLH1*, it is obvious that CIMP cancers share a similar phenotype with sporadic MSI-H. It is interesting that those CIMP+/MSS CRCs are associated with a worse prognosis, while MSI-H CRCs show better prognosis [17] (Fig. 5.1).

The additional events regulating both prognosis and *MLH1* methylation and, thus, MSI status are unclear. Not all researchers in this field have accepted the concept of CIMP. Over the past few years, there has been debate as to whether the CIMP tumors represent a biologically distinct subgroup of CRCs or an artificially selected group from a continuum of tumors showing different degrees of methylation at particular loci. Since original CIMP panel was inadequate to classify CRCs into well-defined subsets, an alternative panel of markers (*CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1*) has been proposed [18]. By this new panel, CRCs distribute bimodal into new CIMP+ and new CIMP- cases, with an even close correlation between CRCs with new CIMP+ and CRCs with *BRAF* mutation. In other words, CRSs with new CIMP+ is almost the same as the sporadic CRC with MSI-H. We have shown that the degrees of promoter methylation at multiple loci in CRC are closely related to the mutational status of *BRAF* and *KRAS*. Since *BRAF* and *KRAS* mutations occur in a mutually exclusive manner, a pathway common to both is critical in developing cancers [19]. The *RAS-RAF-MEK-ERK* signaling pathway is important in apoptosis and particular in anoikis, the process of apoptosis following loss of the epithelial connection to the basement membrane. Failure of anoikis has an important role in developing hyperplastic polyps and serrated adenomas, which are the postulated precursors of CIMP+ colorectal cancers. *BRAF* and *KRAS* mutations interrupt the *RAS-RAF-MEK-ERK* signaling pathway at different levels, impairing normal anoikis [20].

5.4.2 Serrated Pathway

The hypothesis of the "serrated neoplasia pathway," in which serrated polyps (sessile serrated adenomas, hyperplastic polyps, serrated adenomas, and admixed polyps) are the precursors of the sporadic MSI-H CRCs, is supported by the recent finding that 78% of sessile serrated adenomas exhibit *BRAF* mutation. Cancers from this pathway may begin as hyperplastic aberrant crypt foci (ACF), becoming right-sided sessile serrated adenomas, and ultimately develop to MSI-H CRCs (Fig. 5.1). *BRAF* mutation and associated failure of anoikis may be important at least in the early stage of this pathway to form a serrated architecture. The methylator pathway is usually associated with *BRAF* mutation with or without promoter methylation of *MLH1,* resulting in MSI-H or MSS CRC, respectively [21]. It is interesting that there is an association between *MGMT* methylation and *KRAS* mutation in a subset of MSS/CIMP+ cancers. There may be an alternate methylator pathway, without *BRAF* mutation, but rather with the acquisition or maintenance of *KRAS* (G to A) mutation following and the result of the promoter methylation of *MGMT* (Fig. 5.1). It is possible that mutation of *BRAF* with or without promoter methylation of *MLH1* may define one methylator pathway, while the methylation of *MGMT* and *KRAS* mutation could characterize an "alternate methylator" subtype. The precursor lesions for these ultimately "*KRAS* mutant/*MGMT*-methylated" cancers may be adenoma partly being serrated polyp, but this is an area requiring for further

research. Accordingly we proposed the four molecular carcinogenesis pathway of CRC (Fig. 5.1).

5.5 Recent Molecular Classification After Next-Generation Sequencing Era

Gene expression-based subtyping is widely accepted as a relevant source of disease stratification [22]. After emergence of powerful next-generation sequencing, more comprehensive and precise genetic cross-nation analysis was made. Cancer Genome Atlas (TCGA) Research Network has reported integrated genome-wide studies of ten distinct malignancies including colon (COAD) and rectal (READ) adenocarcinomas (Cancer Genome Atlas Research Network, 2012), lung squamous cell carcinoma (LUSC) (Cancer Genome Atlas Research Network, 2012), breast cancer (BRCA) (Cancer Genome Atlas Research Network, 2012), acute myelogenous leukemia (AML) (Cancer Genome Atlas Research Network, 2013), endometrial cancer (UCEC) (Kandoth et al., 2013), renal cell carcinoma (KIRC) (Cancer Genome Atlas Research Network, 2013), and bladder urothelial adenocarcinoma (Cancer Genome Atlas Research Network, 2014) [22]. The subclassification is based on recurrent genetic and epigenetic alterations that converge on common pathways (e.g., *p53* and/or *Rb* checkpoint loss; *RTK/RAS/MEK* or *RTK/PI3K/AKT* activation). Meaningful differences in clinical behavior are often correlated with the single-tissue tumor types, and, in a few case, single-tissue subtype identification has led to therapies that target the driving subtype-specific molecular alteration(s). *EGFR* mutant lung adenocarcinomas and *ERBB2*-amplified breast cancer are two well-established examples [22]. Despite the widespread use, its translational and clinical utility is hampered by discrepant results, likely related to differences in data processing and algorithms applied to diverse patient cohorts, sample preparation methods, and gene expression platforms. Attempt to elucidate intrinsic subtypes of CRC was made elsewhere [23]. Inspection of the published gene expression-based CRC classifications revealed only superficial similarities [24]. For example, all groups identified one tumor subtype enriched for microsatellite instability (MSI) and one subtype characterized by high expression of mesenchymal genes, but failed to achieve full consistency among the other subtypes [23].

5.6 Chemotherapy and Treatment Response

Predictive and prognostic meaning of tumor location is not well understood. Such knowledge may shed light on interactions linking tumor location and treatment response and outcome that may guide personalized therapy. Notably, proximal-sided tumors are more frequently characterized by a host of adverse prognostic

factors, including *BRAF* mutation positivity, MSI (prognostic in stage IV disease), hypermutation, serrated pathway signature positivity, and mucinous histology; conversely, distal-sided tumors more frequently possess gene expression profiles characteristic of EFGR inhibitor-sensitive phenotype (i.e., EGFR/ERBB2 amplified, epiregulin high, and possessing classic chromosomal instability) [25]. The existence of six subtypes of CRC based on the combined analysis of gene expression profiles are suggested and differential response to cetuximab. These subtypes are phenotypically distinct in their DFS and vary in degree of response to cetuximab and standard-of-care chemotherapy. These CRC subtypes are associated with distinctive anatomical regions of the colon phenotype and with location-dependent differentiation states and Wnt signaling activity. Candidate biomarkers that might be developed into clinical qRT-PCR or immunohistochemical assays were identified to classify CRC tumors into one of six subtypes as a guide to assignment of subtype-specific therapeutic agents. With regard to first-line chemotherapy, particular subtypes might show beneficial responses to FOLFIRI in either adjuvant or metastatic settings, whereas in unselected CRC, this treatment did not improve survival in the adjuvant setting. Stemlike-subtype tumors, both in the adjuvant and metastatic settings, as well as inflammatory-subtype tumors in the adjuvant setting, may best be treated with FOLFIRI. Additionally, the transit-amplifying sub-subtypes and the goblet-like subtype will probably not respond to FOLFIRI in the adjuvant setting. Watchful surveillance might spare patients with these forms of disease from the harmful side effects of debilitating and ineffective FOLFIRI treatment. Moreover, and in contrast to the adjuvant setting, the CS-TA or CR-TA subtype might be effectively treated with cetuximab or a cMET inhibitor, respectively, in the metastatic setting [26]. These molecular differences manifest as differential clinical behavior, with right-sided tumors typically displaying worse prognosis. Nevertheless, primary tumor location has not traditionally been included as a stratification criterion in clinical trials, and the influence of tumor location on responsiveness to particular therapies remains incompletely understood. However, primary tumor location could be an important prognostic factor in previously untreated metastatic CRC. Given the consistency across an exploratory set and two confirmatory phase III studies, side of tumor origin should be considered for stratification in randomized trials [27]. Primary tumor location and *KRAS* codon 12/13 mutational status interact on the outcome of patients with metastatic CRC receiving cetuximab- based first-line therapy. Distal-sided primary tumor location might be a predictor of cetuximab efficacy [28]. Also, retrospective analysis of the NCIC CTG CO.17 trial recently reported that tumor location was predictive of treatment benefit. In this population of chemotherapy-refractory patients with *KRAS* wild-type metastatic CRC, adding cetuximab to best supportive care significantly benefitted patients with distal-sided tumors, but has limited benefit in patients with proximal-sided tumors. Furthermore, a significant interaction was observed between tumor location and treatment for progression-free survival. Patients with a proximal-sided primary have more negative prognostic factors and indeed have inferior outcomes compared with those with a distal-sided primary [29]. In the *RAS* wild-type

population of CRYSTAL and FIRE-3, patients with distal-sided tumors had a markedly better prognosis than those with proximal-sided tumors. First-line FOLFIRI plus cetuximab clearly benefitted patients with distal-sided tumors (vs FOLFIRI or FOLFIRI plus bevacizumab, respectively), whereas patients with proximal-sided tumors derived limited benefit from standard treatments [25]. Nibolumab (nivo) showed durable responses and disease control in heavily pretreated patients with dMMR/MSI-H metastatic CRC. Treatment was well tolerated, with no new safety signals (ASCO abstracts).

5.7 Conclusions

At present CRC is understood as a genetic disease, and the attempt of advanced molecular classification is applied to the patients to accomplish personalized medicine. In order to identify molecular classification, examination of several surrogate markers instead of going through precise genetic alterations is desired. CRCs located either proximal or distal to the splenic flexure of the colon have been considered as belonging to the different clinicopathological or physiological categories. Now tumor location in colorectum can possibly become an important surrogate marker to estimate prognosis and [6] to determine the treatment decision including selection of chemotherapy agents for CRC.

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