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Genetics of rectal cancer and novel therapies; primer for radiologists.

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Abstract

Rectal cancer accounts for one-third of newly diagnosed colorectal cancer cases. Given its anatomical location and risk for local recurrence, a multidisciplinary treatment program including surgery, radiation therapy, and chemotherapy has demonstrated improved outcomes in localized disease. Genetic analysis has become part of the standard approach for management of advanced disease and new trials are considering tailored therapies for locally advanced disease. This review describes molecular subsets of colorectal cancer; implications for clinical management, including patterns of metastatic spread and response to therapies; and emerging matched therapies. During the last decade, significant biological differences have been noted based on colorectal cancer primary location and here we focus on rectal cancers and relevant markers for this disease. As more treatment for localized rectal cancer is shifted to the neoadjuvant setting and more targeted regimens are developed for metastatic disease, radiologists will increasingly see patients defined by molecular subsets and their awareness of the genetics of rectal cancer will help further refine our understanding of this disease.

Keywords

rectal cancer; next generation sequencing; targeted therapy; genomics

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States with 50,000 cases in 2017 [1]. Rectal cancer accounts for approximately one-third of newly diagnosed cases and is considered a challenging disease given its anatomical location and risk for local recurrence. Additionally, the incidence of rectal cancer has been rising among younger patients [2]. Seminal work by Fearon and Vogelstein has elucidated the successive genetic changes that underlie transformation from an adenoma to carcinoma in CRC [3,4]. While this basic framework describes cancer development across the large

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Conflicts of interest

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bowel, recent advances in sequencing technology have identified genetic differences between rectal cancers and colon cancers. This review will focus on the genetic alterations underlying rectal cancer development and potential targets for therapy.

Molecular pathogenesis of CRC

Genomic instability has long been recognized as enabling multistep tumor progression [5], and CRC is notable for a high degree of genomic instability. In CRC, this genomic instability can result from three different molecular pathways: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) (Figure 1). Understanding the pathway to tumorigenesis has important clinical implications in rectal cancer as it affects screening recommendations, response to chemotherapy and radiotherapy, and potential targets for matched therapies.

Chromosomal instability pathway

CIN most frequently leads to genomic instability in CRC. CIN occurs in 70% of sporadic CRC, with increasing prevalence in the more distal aspect of the large bowel. CIN involves the gains and losses of whole chromosomes leading to aneuploidy, amplifications, and a high frequency of loss of heterozygosity [6] The CIN phenotype can result from defects in mechanisms that ensure accurate chromosome segregation including dysfunction of the mitotic checkpoint, abnormal centrosome number and function, telomere dysfunction, and failure in DNA damage response machinery [7]. Coupled with the typical karyotypic abnormalities observed in CIN tumors, the accumulation of driver genomic alterations in CRC such as *APC, KRAS,* and *SMAD4* has been historically designated the “chromosomal instability pathway” [7]. However, these mutations are not exclusive to this pathway and can occur also in microsatellite instability high (MSI-H) or CIMP-high tumors. CIN has been associated with young onset CRC, whose incidence has been increasing steadily during the last decades, particularly in the distal colon and rectum [2,8].

Microsatellite instability

In a subset of CRC patients, genetic alterations leading to the development of carcinoma result from defects in the mismatch repair (MMR) proteins, including MLH1, PMS2, MSH2, and MSH6, as well as MLH2, MSH3, PMS1, and Exol. Mutation in one of the MMR genes rendering the protein nonfunctional or hypermethylation of the *MLH1* promoter leading to promoter silencing of MLH1 [9] results in abnormal DNA proofreading after replication, particularly affecting the lengths of short tandem repeats, called microsatellites, within noncoding and coding regions [10]. Asa result, microsatellites are of variable lengths in these tumors, a phenomenon known as microsatellite instability (MSI). Mutation in the MMR genes is often due to a germline alteration, as seen in hereditary non-polyposis colorectal cancer, also known as Lynch Syndrome. In these tumors, genomic instability results from the MSI pathway, and these tumors can harbor numerous mutations, particularly frameshift alterations, in microsatellites across the genome. The prevalence of MSI-H CRC decreases in advanced stages and represents around 20% of cases in stage I-II, 12% in stage III and 4% in stage IV CRC [11,12]. While the prevalence of MSI-H cancers overall is higher in right-sided tumors, those resulting from mutations in the MMR genes

occur with a similar frequency of about 5% across the large bowel [13]. Thus, rectal tumors that are MSI-H are more likely due to Lynch syndrome or sporadic mutations in the MMR genes [13].

CpG island methylator phenotype

Most of the CpG sites are normally methylated in adult cells, however, CpG islands in promoter region of many genes are normally unmethylated [14]. CpG methylation in promoter regions allows regulation of gene expression as methylation leads to transcriptional silencing. As noted above, the MMR gene *MLH1* promoter is often hypermethylated leading to decreased protein expression. Tumors with *MLH1* methylation are microsatellite unstable but develop through a distinct pathway called the CIMP pathway. It is observed in about 15% of tumors [3], particularly in the proximal colon and is infrequent in rectal tumors [15,13].

Clinical implications of molecular subtypes

Evaluation of MMR protein status is recommended for all CRCs. As most MSI-H rectal cancers are due to genetic alterations in the MMR genes, germline assessment of these genes in patients with MMR deficient rectal tumors is important to identify patients who would benefit from genetic counseling and to guide cancer screening.

Patients with MSI-H CRC have a better prognosis in early disease but have worse outcomes for metastatic disease. Recently a series of 62 patients with MSI-H rectal cancers reported a 5-year rectal cancer-specific survival of 100% for stage I and II, 85% for stage III, and 60% for stage IV disease [16]. The pathologic complete response (pCR) rate in 29 patients who received neoadjuvant chemoradiotherapy and underwent surgery was 27.5%, which compares favorably to historical pCR rate in non-MSI-H cohorts. For locally advanced rectal cancer, MSI-H tumors have been associated with increased sensitivity to radiation treatment in preclinical studies [17–19] but large clinical series have not confirmed this increased sensitivity, and some studies have suggested relative radio-resistance [20]. These tumors appear to have more heterogeneous responses to induction chemotherapy and while they can have complete response to treatment, a substantial portion may be chemo-resistant [21].

In more advanced disease, MSI testing is important as it identifies a subset of patients with a high likelihood of response to immune checkpoint inhibitors as described below. Frameshift alterations, which are enriched in MSI-H cancers, are more immunogenic than other classes of genomic alterations [22]. In contrast, CIN, which is much more common in rectal tumors, leads to copy number alterations that are less immunogenic. The copy number alterations, however, can produce potential targets for therapy such as *ERBB2* amplification, described below.

CMS subtypes

In an effort to refine the molecular classification of CRC, the CRC Subtyping Consortium has described a consensus molecular subtype (CMS) classification consisting of four subgroups based on results from six independent transcriptomic-based subtyping systems [23]. These groups are CMS1 (microsatellite instability immune), characterized by

microsatellite instability and immune activation; CMS2 (canonical), characterized by marked WNT signaling activation; CMS3 (metabolic) characterized by metabolic dysregulation; and CMS4 (mesenchymal) characterized by prominent transforming growth factor activation and stromal invasion. In multivariate analysis, CMS4 was associated with worse relapse-free survival and overall survival and CMS1 was associated with poor overall survival after relapse. These molecular subtypes distribute unequally between left- and right-sided tumors. In an analysis of 1603 stage II/III CRC patients treated in the NSABP/ NRG C-07 trial, CMS2 corresponded to 53% and 21% of left- and right-sided tumors, respectively whereas CMS1 corresponded to 11% and 38% of left- and right-sided tumors, respectively (P<.001) [24]. While the CMS classification provides insight into the transcriptional program of CRC, this classification system does not yet impact clinical management.

Specific genomic alterations in rectal cancer

The most recurrent genomic alterations in CRC involve the *APC*, *TP53*, and *KRAS* genes [25,26], and these genes are also the most commonly mutated in rectal cancers. However, there are variations in gene alterations by tumor locations – *APC* and *TP53* mutations are more common in the rectum than in the proximal colon (78% versus 70% for *APC*; 81% versus 65% for *TP53*) and *KRAS* mutations are much less common in the rectum than in the proximal colon (39% versus 65%) [26]. Mutations in the V600 hotspot of *BRAF* rarely occur in rectal tumors. These differences in genomic alterations affect response to therapy, patterns of metastatic spread, and potential targets for matched targeted therapies.

Response to therapies

The treatment of locally advanced rectal cancer is multidisciplinary. Standard treatment often includes chemoradiotherapy, total mesorectal excision and adjuvant chemotherapy [27–29]. With this treatment sequence the incidence of local recurrence has been consistently reported below 10% and, in 15 to 27% of patients, no residual viable tumor cells are detected in the resected specimen [30,31]. A variation of this paradigm is the total neoadjuvant therapy approach, which considers chemotherapy before surgery administered either before or after neoadjuvant chemoradiotherapy [32,33]. This strategy aims for early treatment of micrometastatic disease and to increase pCR rate. In patients with clinical complete response after neoadjuvant treatment, non-operative management has also been attempted in order to avoid the impairment of quality of life associated with total mesorectal excision [34,35]. The identification of predictive biomarkers to correctly select patients for these different strategies has been challenging. As noted above, the presence of microsatellite instability may affect response to these standard therapies, potentially sensitizing to radiation treatment and leading to a larger degree of heterogeneity in response to chemotherapy. New studies are evaluating the potential of induction immunotherapy in patients with locally advanced MSI-H tumors based on the activity of this approach in advanced disease and in the neoadjuvant setting [36]. However, MSI-H tumors represent a minority of rectal cancers. A systematic review did not find any pathological factors, imaging modalities, or molecular factors consistently associated with pCR following neoadjuvant chemoradiotherapy [37]. Studies included in this review evaluated molecular biomarkers such as gene signatures by microarray, *KRAS* and *TP53* mutations, single

nucleotide polymorphisms, and protein expression profile. In a more recent multicenter study including 292 patients with stage II/III rectal cancer, the pCR rate after neoadjuvant treatment in *KRAS* mutant and *KRAS* wild type tumors was 15% and 34%, respectively [38]. *KRAS* mutation remained independently associated with a lower pCR rate on multivariate analysis (OR 0.34; 95% CI 0.17-0.66, P=0.01). Figure 2 shows serial images from a patient treated at our institution for locally advanced *KRAS* mutant rectal cancer who received induction chemotherapy followed chemoradiotherapy with modest clinical benefit. In patients with stage I tumors, *KRAS* mutations have also been associated with higher risk of recurrence after local excision [39].

Patterns of metastatic involvement

When they metastasize, rectal tumors more commonly spread to the lungs and bones and less commonly involve the peritoneum/omentum or gynecologic organs compared to right-sided and left-sided primary colon cancers. Relative flow of first site of metastasis from colorectal cancer according to different primary tumor location is described in Figure 3 [26]. The higher incidence of lung involvement has been attributed to anatomic reasons. A distal rectal tumor may metastasize initially to the lungs because the inferior rectal vein drains into the inferior vena cava, bypassing the portal venous system [40]. This is seen despite a lower frequency of *KRAS* mutations, which is associated with lung involvement [41,42]. Peritoneal involvement is commonly seen in MSI-H cancers that metastasize, particularly those that develop through the CIMP pathway, and in *KRAS*-mutant tumors, which are both more common in proximal colon cancers than in rectal cancers.

Potential targeted therapies

The majority of rectal cancers are wild-type for the *RAS* genes and may therefore be targeted with the anti-EGFR antibodies cetuximab and panitumumab since *RAS* mutations are associated with resistance to these targeted agents [43,44]. Expression of the EGFR ligands, amphiregulin and epiregulin, is known to vary across the large bowel and predicts for response to EGFR inhibitors [45]. Anti-EGFR antibodies have also been tested in locally advanced rectal cancer, but their addition to standard therapies has not been able to improve the pCR rate [46–50].

Because of the high frequency of copy number alterations, gene amplifications are more common in distal tumors. *ERBB2* (also known as *HER2)* amplification is emerging as a target. In a retrospective study in which 365 colorectal tumors were analyzed by in-situ hybridization, the prevalence of *HER2* amplification was higher in rectal cancer (10.4%) compared to left-sided tumors (3.6%) and right-sided tumors (2.9%) (P=.013) [51]. The prognostic features of HER2 overexpression in rectal cancer was specifically studied in a retrospective cohort by Meng et al. [52]. In this cohort, HER2 overexpression by immunohistochemistry (IHC 3+ or IHC 2+ and FISH *HER2*-positive) was found in 115 out of 717 rectal tumors (16%). In the subgroup of HER2-positive patients the 5-year overall survival was significantly shorter than those of HER2-negative patients (63.5% vs. 73.9%, P=.013). Recent trials targeting HER2 with the combinations of trastuzumab-lapatinib and trastuzumab-pertuzumab have shown encouraging activity in metastatic *HER2*-amplified CRC with response rates of about 30% [53,54].

BRAF V600E mutation is rare in rectal cancer and seen in less than 1% of cases. While infrequent, testing for *BRAF* V600E is important to guide the use of EGFR inhibitors and of potentially matched targeted therapy. This mutation has been associated with poor prognosis and lack of response to anti-EGFR monoclonal antibodies [55,56]. Currently there are no FDA-approved targeted therapies for V600 *BRAF*-mutant CRC, however, combinations of a selective RAF inhibitor and EGFR antibody have shown promising preliminary activity. The National Comprehensive Cancer Network (NCCN) guidelines include the triplet of the RAF inhibitor vemurafenib, EGFR antibody cetuximab, and irinotecan for improved progression free survival compared to cetuximab and irinotecan in *BRAF* V600E CRC [57] and the triplet combination of the RAF inhibitor encorafenib, the MEK [the protein target of BRAF] inhibitor binimetinib, and cetuximab recently received FDA breakthrough designation for the treatment of *BRAF* V600E CRC after progression through standard therapy based on a response rate of about 50% in a safety lead-in cohort of 29 patients [58].

As noted above, up to 5% of rectal tumors are MSI-H, most commonly due to mutations in the MMR genes. The presence of microsatellite instability opens an important treatment option with immune checkpoint blockade. Response rates to single agent anti-PDl inhibitors range from 31-52% and combination treatment with anti-PDl and anti-CTLA4 inhibitors achieves a response rate of 55% [59–61]. These immunotherapy strategies have been associated with durable benefit in responders. Figure 4 shows serial images from a patient with MSI-H sigmoid colon cancer with local recurrence who was treated with an anti-PDl inhibitor with a clinical complete response.

Conclusion

Rectal cancer is clinically distinct from colon cancer as localized disease presents a unique challenge requiring precise radiographic assessment of disease extent and careful coordination of treatment between surgical, radiation, and medical oncologists. An understanding of the genetics underlying CRC development provides a framework to further interpret the behaviors of rectal cancers. Molecular characteristics leading to clinical variations occur across the colorectum. Tumors located in the rectum share most genomic features with left-sided colon neoplasms including high rate of CIN and low frequency of adverse prognostic biomarkers such as *BRAF* V600 and *RAS* mutations. Rectal tumors exhibit a higher prevalence of copy number alterations, including clinically targetable alterations like *HER2* amplification, and lower prevalence of MSI-H tumors secondary to MLH1 promoter methylation. New matched therapies have the potential to improve outcomes for metastatic disease and tailor treatment in localized disease. However, the incorporation of novel systemic therapies has not been straightforward and drugs that have demonstrated a benefit in overall survival in metastatic CRC have failed to improve outcomes in localized rectal cancer, so more work remains to be done. As radiologists are aware of the genetics of rectal cancer, their insights will help further refine our understanding of this disease.

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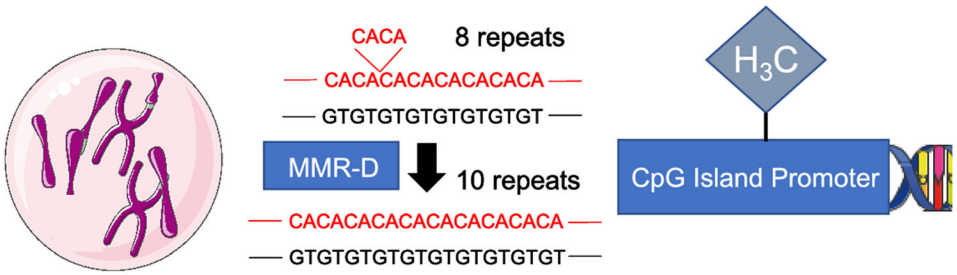

	CIN	MSI	CIMP
Hallmarks	Aneuploidy Amplifications LOH	Mutation MMR genes High mutation burden	*MLH1* promoter methylation
Prevalence	70-80%	15-20%	10-15%
Frequent tumor location	Left-sided/Rectum	No predilection	Right-sided
Frequent GA	APC KRAS SMAD4	TGFBR2 BAX	BRAF V600 CDKN2A methylation

Figure 1. Genomic instability pathways in colorectal cancer.

Abbreviation: CIN, chromosomal instability; MSI, microsatellite instability; CIMP, CpG island methylator phenotype; LOH, loss of heterozygosity; MMR, mismatch repair; GA, genomic alteration.

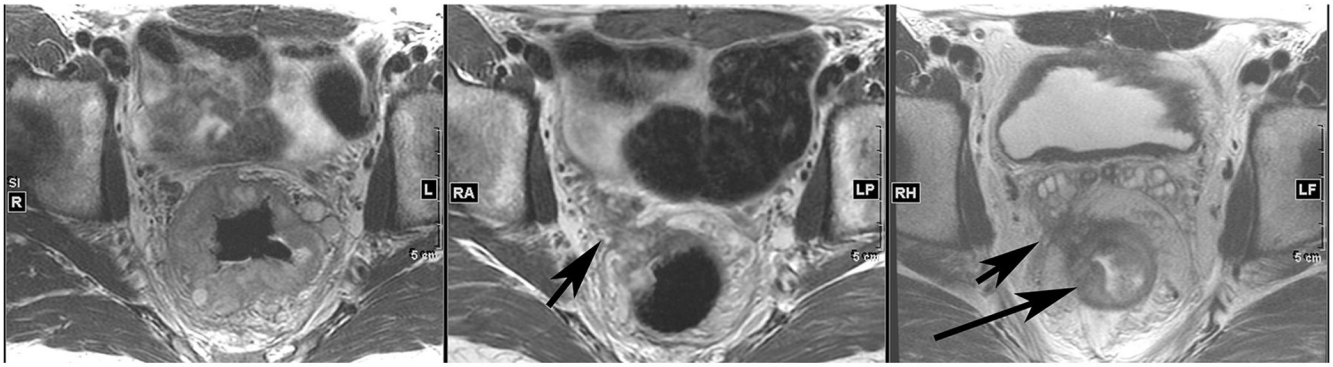

Figure 2. Serial rectal MRIs during total neoadjuvant therapy for locally advanced KRAS G12D rectal cancer.

(Left) Baseline axial T2-weighted image shows large circumferential tumor with surrounding lymph nodes. (Center) Post-induction chemotherapy axial T2-weighted image shows moderate response with decreased tumor size but persistent tumor extending through mesorectum to contact the right seminal vesicle (arrow). (Right) Post-chemoradiotherapy axial T2-weighted image shows new edema of the rectal wall (long arrow) with little to no decrease in tumor in prior location (arrow).

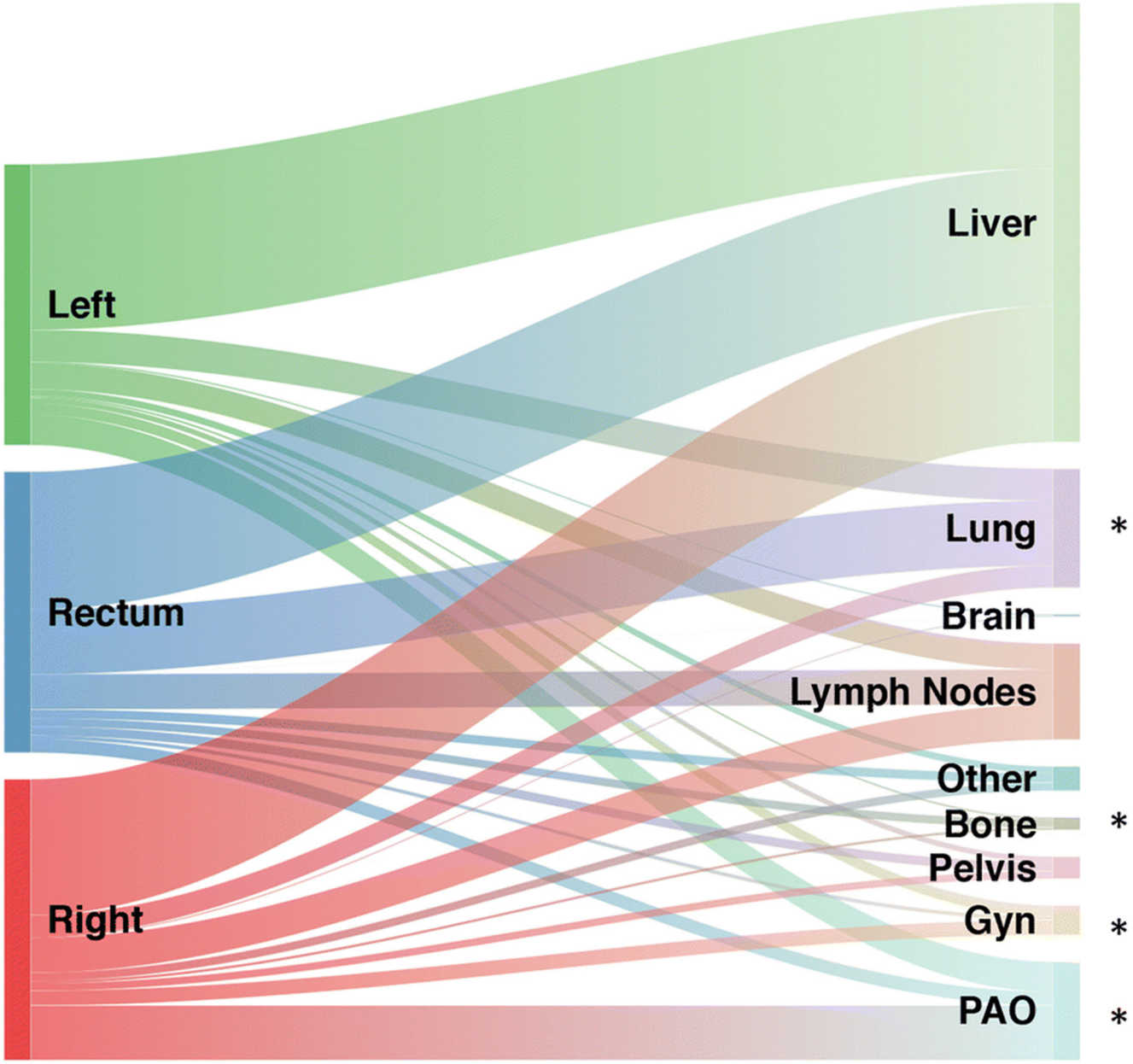

Figure 3. Sankey diagram describing relative flow of first site of metastasis from colorectal cancer according to different primary tumor location.

Abbreviation: Gyn, ovaries, fallopian tubes, uterus, cervix, and vagina; PAO, peritoneum, abdominal wall or omentum. Asterisk indicates a statistically significant difference (P<0.05).

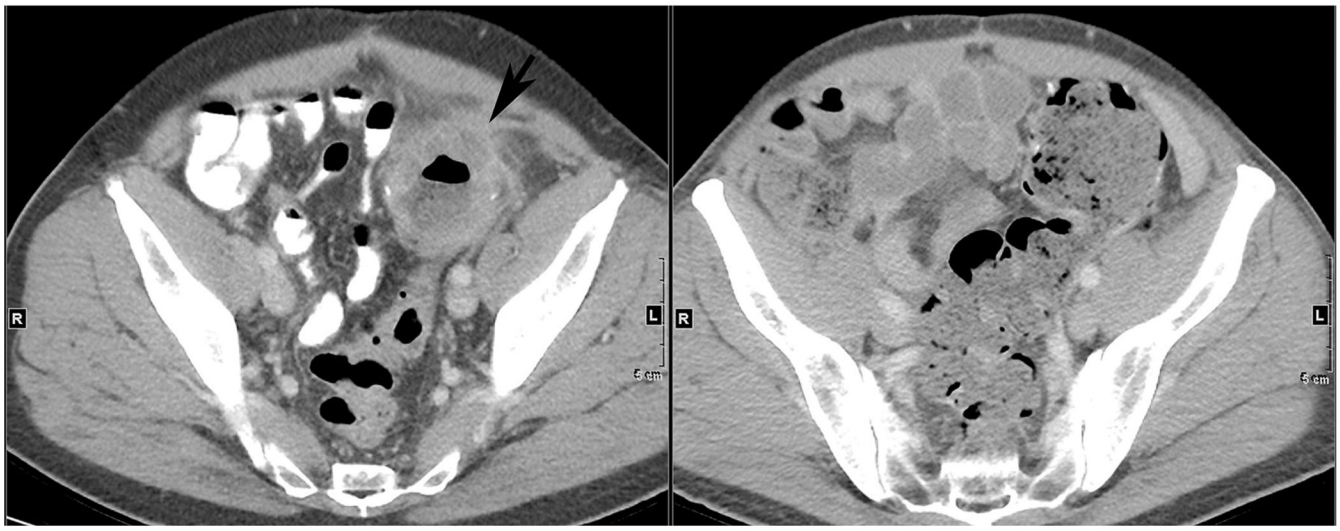

Figure 4. CT imaging from a patient with resected MSI-H sigmoid colon cancer with a local recurrence.

(Left) Axial contrast enhanced CT scan at level of anastomosis revealing circumferential recurrent tumor (arrow). (Right) Axial contrast enhanced CT scan at level of anastomosis post Pembrolizumab treatment for 10 weeks showing complete normalization of anastomosis.