Surgery, Perioperative Treatment and Prognostic Factors
Based on Genetic and Epigenetic Alterations in Colorectal Cancer

KIHICHI SUGIMOTO*, KAZUHIRO SAKAMOTO*

*Department of Coloproctological Surgery, Juntendo University Faculty of Medicine, Tokyo, Japan

In this review, we summarize the current status and provide future perspectives on surgery, perioperative treatment, and prognostic factors based on genetic and epigenetic alterations in colorectal cancer (CRC).

Surgery is still the mainstay of treatment for CRC for definitive management and potential cure at an early stage, and effective palliation in advanced cases. Local control and survival have significantly improved in surgical cases because the indication for surgery and perioperative treatment has changed. Based on the results of some randomized control trials, laparoscopic surgery is now considered to be a standard treatment option for advanced colon cancer. The most recent development is robotic surgery using the da Vinci® surgical system, which consists of a patient-site cart, a surgeon console, and a vision system. This system provides 3 dimensions high definition (3D HD) vision of a clear and magnified operative view and converts the surgeon’s hand movements into smaller, more precise movements of tiny instruments inside the body. Thus, da Vinci® technology allows surgeons to perform complex procedures through a few small wounds, similarly to conventional laparoscopic surgery.

Genetic effects such as mutations and chromosome instability involve changes in the DNA sequence of a gene, whereas epigenetics refers to potentially heritable changes that are linked to DNA methylation or histone methylation, but do not involve changes in the DNA sequence. Genetic and epigenetic changes are linked with each other and can lead to cancer development and progression. Many recent studies have examined the relationships of these changes to diagnosis, treatment and prevention of CRC.

Key words: colorectal cancer (CRC), surgery, perioperative treatment, genetic alteration, epigenetic alteration

Introduction

Significant recent improvements in surgery and perioperative treatment for colorectal cancer (CRC) have led to better short- and long-term outcomes. Many studies have also identified prognostic factors based on genetic and epigenetic alterations in CRC. These alterations are linked with each other and can lead to cancer development and progression. Therefore, understanding of genetic and epigenetic alterations is crucial for further improvement of outcomes in CRC. In this review, we summarize the current status and provide future perspectives on surgery, perioperative treatment, and prognostic factors based on genetic and epigenetic alterations in CRC. Based on these results, laparoscopic surgery is now considered to be a standard treatment option for advanced colon cancer.

Surgery and perioperative treatment for colorectal cancer

Surgery is still the mainstay of treatment for CRC for definitive management and potential cure at an early stage, and effective palliation in advanced
cases. Local control and survival have significantly improved in surgical cases because the indication for surgery and perioperative treatment has changed.

1. Colorectal cancer incidence and mortality in Japan

In Japan, the cancer incidence has increased rapidly. The incidence of CRC is twofold higher in men than in women, and among all major cancers the incidence is second in men and first in women. Thus, CRC is one of the major causes of cancer death in Japan, with the mortality of CRC fourth in men and highest in women among major cancers. However, in the Health at a Glance 2011 Organisation for Economic Cooperation and Development (OECD) Indicators, Japan also had the highest survival rate (68%) in CRC among OECD countries from 2004 to 2009.

2. General rules of treatment for colorectal cancer

The goals of the guidelines of the Japanese Society for Cancer of the Colon and Rectum (JSCCR) are as follows: 1. To disseminate standard treatment strategies for CRC; 2. To eliminate disparities among institutions in terms of treatment; 3. To eliminate unnecessary treatment and insufficient treatment; and 4. To deepen mutual understanding among healthcare professionals and patients. The Japanese classification system for CRC is shown in Table 1. T status indicates the tumor depth (Tis to T4b), and T4b means that the tumor has invaded adjacent organs. N status refers to lymph node metastasis (LNM) (N0 to N3). In CRC, the stage is divided into 6 categories: stages 0, I, II, IIIa, IIIb and IV.

Treatment strategies for preoperatively diagnosed Stage (cStage) 0 to III CRC are shown in Table 2. Lymph node dissection (LND) is unnecessary for Tis (M) and some T1 (SM) cases because these cancers do not have LNM. D refers to LND; therefore, D0 cases require bowel resection without LND. Endoscopic treatment is suitable for Tis and some T1 cases, and includes polypectomy, endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). Indication criteria for endoscopic resection are (1) intramucosal carcinoma or carcinoma with slight submucosal invasion, (2) size is unimportant, and (3) any macroscopic type. In contrast, LND is necessary for some T1 cases (SM deep) and deeper cancer (T2–T4). D2 dissection is necessary for SM deep cancer because the incidence of LNM is approximately 10%. Standard treatment for these cancers is tumor resection with regional LND. Two decades ago, open procedures were performed; however, recently we have performed minimally invasive surgery, including laparoscopic and robotic surgeries.

Treatment strategies for Stage IV CRC are shown in Table 3. These strategies are more complicated because of the need for treatment of the primary tumor and distant metastasis. Stage IV CRC is associated with synchronous distant metastasis, such as to the liver, lung, peritoneum, brain, distant lymph nodes, or other organs (bone, adrenal gland, spleen). If both distant metastases and primary tumor are resectable, curative resection of the primary tumor is performed and resection of distant metastases is considered. If distant metastases are resectable, but the primary tumor is
unresectable, resection of the primary tumor and distant metastases is not recommended and another treatment method should be considered. If distant metastases are unresectable, but the primary tumor is resectable, the indication for resection of the primary tumor should be considered based on the clinical symptoms of the primary tumor and the effect on prognosis.

### 3. Minimally invasive treatment for colorectal cancer

There are many short-term advantages to the patient of laparoscopic colectomy (LAC) compared with open colectomy (OC), including smaller incisions, which is the major benefit, reduced hemorrhage, less pain, and shorter recovery time. However, long-term outcomes in laparoscopic surgery were unclear until the JCOG 0404 Japanese multicenter randomized clinical trial (RCT), which showed that 3- and 5-year overall survival rates after laparoscopic surgery were equivalent to those after open surgery group. Based on these results, laparoscopic surgery is now considered to be a standard treatment option for advanced colon cancer. In other major RCTs of laparoscopic-assisted surgery for CRC, such as the Cost trial in the US, CLASSIC trial in the UK, and COLOR trial in the EU, there were also no differences in overall and disease-free survival rates, compared to open surgery.

Up to 2014, we had performed LAC in 1,104 cases of CRC. Long-term outcomes of LAC were compared
with those of OC using propensity score (PS)-matching of cases because of the major differences in indications for the two procedures. After PS matching, univariate analysis showed no significant differences in clinicopathological factors between LAC and OC cases. Before PS matching, recurrence-free survival after LAC was significantly better than that after OC (hazard ratio = 0.59, 95% CI: 0.35-0.98, p = 0.04) (Figure 1A). However, after PS matching, there was no significant difference in recurrence-free or cancer-specific survival (Figure 1C, D), indicating that long-term outcomes of LAC are equivalent to those of OC.

There are, however, some concerns in laparoscopic surgery for rectal cancer due to technical difficulties. Autonomic nerve-preserving operations for...
rectal cancer are required to maintain urinary and male sexual functions without reducing the radical effect of therapy. Sexual dysfunction after surgery of the rectum is a serious complication in male patients, and total mesorectal excision (TME) with autonomic nerve-preserving surgery is an important procedure for locally advanced rectal cancer. A high resolution laparoscopic view enables identification of important membranes and nerves that allows preservation of urinary and sexual function without limiting curability. The most recent development is robotic surgery using the da Vinci® surgical system, which consists of a patient-site cart, a surgeon console, and a vision system. This system provides 3 dimensions high definition (3D HD) vision of a clear and magnified operative view and converts the surgeon's hand movements into smaller, more precise movements of tiny instruments inside the body. Thus, da Vinci® technology allows surgeons to perform complex procedures through a few small wounds, similarly to conventional laparoscopic surgery.

4. Perioperative treatment for advanced colorectal cancer

Neoadjuvant therapy is an important treatment option for curative resection in laparoscopic surgery for locally advanced rectal cancer. The latest National Comprehensive Cancer Network (NCCN) Guidelines recommend preoperative chemoradiotherapy (CRT) or systemic chemotherapy for such cases. Preoperative CRT has been commonly used, but systemic chemotherapy is becoming more common because CRT does not reduce the cumulative incidence of distant metastases. A case of locally advanced rectal cancer treated with neoadjuvant chemotherapy with an oxaliplatin-based regimen is shown in Figure–2. Tumor reduction of 30% and a longer distance on the anal side without severe adverse events were achieved after this treatment. Consequently, laparoscopic curative resection with anal preservation was achieved because of effective chemotherapy before surgery. Therefore, preoperative treatment is important and is becoming more common for locally advanced rectal cancer.

Figure–2  A case with locally advanced rectal cancer treated with neoadjuvant chemotherapy with an oxaliplatin–based regimen
Tumor reduction of 30% and a longer distance on the anal side without severe adverse events were achieved after this treatment. Consequently, laparoscopic curative resection with anal preservation was achieved because of effective chemotherapy before surgery. Pre–treatment findings: T3 N1 M0 cStage IIIa, XELOX therapy; 4 courses, Operation: Laparoscopic intersphincter resection + bilateral lymph node dissection, Pathological findings: tub2 ypT1b/SM ly0 v0 N0 (0/25) ypStage I 6), Pathological response: Grade 1b 6) (Watanabe T, Muro K, et al: Int J Clin Oncol, 2018; 23: 1-34 10)
Prognostic factors based on genetic and epigenetic alterations

Genetic effects such as mutations and chromosome instability involve changes in the DNA sequence of a gene, whereas epigenetics refers to potentially heritable changes that are linked to DNA methylation or histone methylation, but do not involve changes in the DNA sequence. Epigenetic changes may involve methylation of bases that represses gene activity through tight packing of chromatin. Genetic and epigenetic changes are linked with each other and can lead to cancer development and progression (Figure-3). Therefore, many recent studies have examined the relationships of these changes to diagnosis, treatment and prevention of CRC.

1. Genetic alterations

Many genetic alterations have been identified in CRC, and understanding the heterogeneity of these changes is important clinically with regard to responsiveness to anticancer drugs and molecular-targeted drugs. In 2015, President Obama announced the launch of the Precision Medicine Initiative, as a bold new research effort to revolutionize health and treatment of disease. Precision medicine considers individual differences in genes, environments, and lifestyles. In CRC, precision medicine depends on the molecular features, as defined in the consensus molecular subtypes of CRC published in Nature Medicine in 2015. A network-based study of the associations among six CRC classification systems identified four robust consensus molecular subtypes (CMS1 to CMS4) with significant interconnectivity (p < 0.001, hypergeometric test). There were also associations between the CMS groups and variables such as overall and relapse-free survival. Therefore, there is a need to understand how molecular features affect individual outcomes and drug responses in performance of precision medicine.

RAS is an important gene in treatment of CRC, since RAS mutations can lead to constitutively activated Ras proteins. In tumors with wild-type RAS, anti-epidermal growth factor receptor (EGFR) drugs can inhibit EGFR signaling to block tumor growth, metastasis and angiogenesis, and promote cell apoptosis. In contrast, in tumors with RAS mutations, these drugs are ineffective because EGFR signaling may be permanently activated. The FIRE-3 phase III trial showed that an anti-EGFR drug (erbitux) gave significantly better overall survival (OS) in patients with KRAS or all RAS...
wild-type tumors, compared with an anti-vascular endothelial growth factor (VEGF) drug (bevacizumab)\textsuperscript{20}. However, in RAS-mutant tumors, the difference in OS disappeared, showing the lack of efficacy of the anti-EGFR drug in these tumors. Based on these results, anti-EGFR drugs are recommended for RAS wild-type CRC.

Recently, tumor location has been shown to be an important prognostic and predictive factor in CRC\textsuperscript{25}. In general, right-sided tumors are hyper-mutated, leading to a poor prognosis, whereas left-sided tumors are associated with EGFR signaling, and thus, are likely to have a better response to anti-EGFR drugs. This difference arises embryologically\textsuperscript{26}. Right-sided colon carcinomas (RCCs) are located in the part of the colon derived from the embryologic midgut, which includes the proximal two-thirds of the transverse colon, ascending colon and cecum. Left-sided colon carcinomas (LCCs) lie within the colon derived from the embryologic hindgut, which includes the distal third of the transverse colon, splenic flexure, descending colon, sigmoid colon and rectum. Clinical studies in patients with metastatic CRC are now evaluating the impact of the primary colonic tumor location on the response to treatment, with a focus on biologics.

Genetic alterations as biomarkers are also important in immunotherapy. Three targeted therapies are currently used for metastatic CRC: immune checkpoint inhibitor (anti–PD–1) for microsatellite instability (MSI)–high tumors, EGFR blockade for left–sided KRAS and NRAS wild–type tumors, and inhibition of VEGF or its receptor VEGFR for unspecified tumors (Table 4). Recent studies have shown an increased mutational burden created by neoepitopes responsible for the immune response. Most significantly, tumors with MSI have significant upregulation of immune checkpoint proteins, including PD–1 and PD–L1, enabling them to survive. A follow–up clinical trial showed the utility of MSI status as a predictive marker for response to PD–1 blockade in Stage IV cancer that was unresponsive to other chemotherapies\textsuperscript{27}. In this trial, 11 patients with mismatch (MMR)–deficient (dMMR) CRC and 21 with MMR-proficient CRC were treated with pembrolizumab, an anti–PD–1 antibody. MSI was a significant predictor of the immune–related objective response (40% in dMMR CRC, 71% in dMMR non-CRC, 0% in MMR-proficient CRC) and of immune-related progression–free survival (78%, 67%, and 11%, respectively). Whole–exome sequencing of tumor tissue revealed an average of 1,782 somatic mutations in cancers with MSI (578 were predicted to result in neoantigens) compared to only 73 somatic mutations in cancers without MSI.

2. Epigenetic alterations

Epigenetics is the study of heritable changes in gene function that do not involve changes in DNA sequences\textsuperscript{19}. Epigenetic alterations are largely based on changes in methylation of DNA bases with gene activity reduced through tighter packing of chromatin. Such changes in tumor suppressor genes can lead to rapid development and progression of cancer. Inactivation of tumor suppressor genes may occur through mutation, chromosomal deletion, and DNA methylation; and unusual cell growth due to DNA methylation of these genes has been reported in most types of cancers\textsuperscript{28}. In humans, the major causes of DNA methylation are aging and chronic inflammation, and both can cause cancer. Therefore, an understanding of DNA methylation is important in diagnosis, treatment and prevention of cancer.

In diagnosis, tumor, blood, urine and stool can be used for early detection of cancer, based on
predictive or prognostic biomarkers\textsuperscript{29}. For treatment, demethylating agents have been developed for some types of leukemia, such as myelodysplastic syndrome (MDS)\textsuperscript{30}. Concerning DNA methylation, current research is aimed at identifying epigenetic signatures that could be used for CRC diagnosis, staging, tendency for metastasis, prognosis, and response to treatment. The CpG island methylator phenotype (CIMP), characterized by widespread promoter methylation, is associated with MSI and BRAF mutation in CRC\textsuperscript{31}. The independent effects of CIMP, MSI and BRAF mutations on prognosis are uncertain. In colon cancer, a CIMP-high status seems to be an independent predictor of low mortality, while BRAF mutation is associated with high mortality\textsuperscript{31}. Therefore, it is important to recognize that colorectal neoplasms are heterogeneous in genetic and epigenetic alterations.

Liquid biopsy is defined as sampling and analysis of non-solid biological tissue, primarily blood\textsuperscript{32}. As for traditional biopsy, this technique is mainly used for diagnosis and monitoring of a disease such as cancer, with the added benefit of being largely non-invasive. Therefore, it can be performed more frequently, which allows better tracking of tumors and mutations over time. In our investigation of p14 gene DNA methylation in plasma samples, we extracted DNA and treated this DNA with bisulfite conversion. The methylation status of the p14 gene was then measured with quantitative methylation-specific polymerase chain reaction (PCR). Patients with lung metastasis had significantly greater methylation of p14 compared to those without lung metastasis (Figure-4). This result indicates that the p14 methylation status in plasma samples may be a prognostic marker in patients with Stage IV CRC.

Conclusions

Minimally invasive surgery with appropriate perioperative treatment can achieve favorable short- and long-term outcomes in CRC. Understanding of genetic and epigenetic alterations as

![Figure-4](Image)
biomarkers for molecular–targeted therapy is important for effective precision medicine in this cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

References