Colonoscopy, Biomarkers, and Targeted Therapy in Colorectal Cancer

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ABSTRACT

This review highlights the most versatile diagnostic test of colonoscopy for CRC. It remains the gold standard diagnostic tools for CRC, and to prevent CRC by screening and removing the polyp or premalignant lesions. This work also provides the most promising biomarkers, which highlights the application of the novel biomarkers in conjunction with clinical and pathologic features have allowed for more individualized approaches and targeted therapy to patients with CRC.

CRC is the third most common cancer diagnosed, and the second leading cause of cancer-related deaths worldwide. With a population totaling 273,523,621 people, Indonesia has an estimated of 396,914 new cases of all cancers and 234,511 cancer-related deaths. Among those cancer cases, an estimated of 34,189 new CRC cases and 17,786 CRC deaths occurred in 2020. Most of CRC cases were located in the rectum compared to those in the distal colon or proximal colon. Clinical signs, symptoms and therapeutic approaches vary, depending on the stage and the location of CRC. Those cancer locations are different in terms of their associated molecular alterations.

Biomarker tests of tumor tissue from colonoscopy biopsy can help doctors to select a specific CRC treatment, and the tests can be used to determine prognostic value, predictive factors and the targeted therapy. Targeted therapies are recommended for advanced or mCRC patients with KRAS/NRAS/BRAF mutated or wild-type tumors, HER2-amplified tumors, and NTRK gene fusion-positive, while immunotherapy is only offered for tumor with MSI-High (dMMR) status. The biomarkers and targeting approaches against colorectal CSCs are being developed and will be quite challenging.

***Keywords**: Colonoscopy, colorectal cancer, biomarkers, cancer stem cells, targeted therapy.*

INTRODUCTION

There were an estimated 19.3 million new cases of all cancers and approximately 10 million cancer-related deaths worldwide in 2020. Colorectal cancer (CRC) is the third most common cancer, and the second leading cause of cancer deaths among all cancers worldwide, with an estimated of 1,931,590 new CRC cases (10% of all cancers), and 915,880 colorectal cancer deaths (9.2% of all cancer deaths) in 2020. With a population totaling 273,523,621 people, Indonesia has an estimated of 396,914 new cancer cases and 234,511 cancer-related deaths. Among those cancer cases an estimated of 34,189 new CRC cases (8.6% of all cancers), and 17,786 CRC deaths (7.6% of all cancer deaths) occurred in 2020. Other countries with increasing populations also experience similar phenomena.[1-3] Most of CRC cases were located in the rectum (74.6%) compared to those in

the distal colon (18.8%) or proximal colon (6.6%).[3,4] More than 50% of CRC patients will develop metastasis, and approximately 25% of them present with metastasis at initial diagnosis. Clinical signs and symptoms vary depending on the location and the stage of the tumor. Those locations are also different in terms of their associated molecular alterations.[3,4]

Colonoscopy and biopsy of the tumor tissue are important in CRC, and it has become a very popular method for primary CRC screening. It allows for early-stage cancer detection and polyps detection and removal. Sporadic CRCs may develop from adenomatous polyps and from sessile serrated lesions.[5,6]

Molecular testing of CRC from tumor tissues or biomarkers has important implications for the selection of treatment. The testing can also be used to determine prognostic value for molecular predictive factors and targeted therapy. Cancer locations, whether the tumor is at proximal colon, distal colon or rectum, are also different in terms of their associated molecular alterations and response with chemotherapy.[3,4,7-9]

CRC PREVENTION

Most polyps identified can be managed by conventional polypectomy, and they do not pose a significant challenge for resection to an adequately skilled and trained endoscopist.[10] The main endoscopic techniques that are available for removal of colon polyps, or early cancer and precancerous growths are as follows: a) Polypectomy, b) Endoscopic Mucosal Resection (EMR) and c) Endoscopic Submucosal Dissection (ESD).[10,11] Colonoscopy is a 1-step CRC screening test, and it is the most appropriate screening test and diagnosis of CRC.[5] With appropriate training, ESD is preferred over EMR as the first-line therapy for resection of colorectal polyps, without restricting to lesion greater than 20 mm and those with high suspicion of submucosal invasion.[11]

CLINICAL MANIFESTATION

Clinical signs and symptoms vary based on the location, the tumor size and the stage of CRC.[3,4] CRC may not cause symptoms in the early stages. If it does, the symptoms may include a change in bowel habits, such as diarrhea, constipation or a change in the consistency of the stool, and these symptoms can last for more than a few days. The change in bowel habits can also induce feeling that the bowel does not empty properly, or result in blood in the stool from the rectum (hematochezia) that is dark brown or bright red in colour, abdominal pain, cramping, bloating, feeling full, fatigue or tiredness, a decrease in appetite, unexplained iron deficiency anemia, and unexplained weight loss. CRC should be classified into several subgroups defined according to tumor location, rather than as a single entity, whether at proximal, distal colon or rectum. In clinical practice, different manifestations, molecular alterations and survivals have been observed in CRC patients with tumor originating from different sub-sites of the colorectum.[3,4,7,12-16]

Tumor location plays a critical role in predicting survival. Right colon cancer patients or proximal colon cancer had worse survival especially in the subgroups including stage III or IV disease.[3,4,12,14] Tumors in the proximal colon or right colon rarely cause symptoms of obstruction. Stool is relatively liquid as it passes through the ileocecal to the cecum and ascending colon. Tumor usually forms ulceration, chronic occult bleeding, microcytic hypochromic anemia due to iron deficiency, fatigue and weight loss. Stool has become more shaped as it passes through the transverse colon to the splenic flexure, descending colon and sigmoid colon (distal colon). Tumor in the distal colon can interfere with the passage of stool causing symptoms of abdominal pain or cramps, altered bowel habit, decreased stool caliber, or overflowed diarrhea. In addition, it sometimes causes symptoms of obstruction and perforation. Cancer located in the rectum will result in hematochezia, tenesmus, small-caliber stools due to narrowing of the rectum caused by the tumor. CRCs spread to other parts of the body by direct extension into adjacent structures and metastasis through the lymphatics and blood vessels. The favoured metastatic sites of CRC are lymph nodes, liver, lung, brain, bone, and peritoneum. Patient with metastatic CRC (mCRC) to the liver may have signs of right upper quadrant abdominal pain,

ascites, jaundice, or symptoms and signs from cancer spread to the lungs. Other symptoms include those from local invasion or perforation to bladder or vagina.[7,12-16]

DIAGNOSTIC EVALUATION

Physical Examination

Physical examination findings can be very nonspecific (e.g., fatigue, weight loss) or normal, early in the course of colon cancer. The examination is performed with specific attention to the abdominal mass, especially at the right and left iliac fossa, right upper abdomen, as well as to possible metastatic lesions, including enlarged lymph nodes, hepatomegaly, and others. Examination includes the use of digital rectal examination (DRE). Patients with proximal colon cancer, especially at the cecum may have abdominal mass, particularly at the right iliac fossa, while patients with sigmoid cancer will have palpable mass at the left iliac fossa. Common signs and symptoms of emergencies due to complications of CRC should be examined, such as peritonitis from perforation, or obstruction. Other signs, such as jaundice, hepatomegaly, ascites, may occur with liver metastasis.[7, 12-17]

Colonoscopy and Biopsy

Certain screening modalities, such as colonoscopy, sigmoidoscopy, CT colonography and to a lesser extent stool-based testing, will detect advanced adenomatous polyps, whereas colonoscopy is optimal for the detection of sessile serrated lesions (SSLs). Endoscopic removal of polyps reduces CRC incidence and CRC mortality.[5] Colonoscopy is regarded as the gold standard diagnostic technique for colorectal tumor detection to determine precisely the location of the tumor.

Pathology and Biomarker Test

Tumor biopsy remains the gold standard for initial pathologic diagnosis and molecular testing in CRC. Fresh tumor tissue is obtained from colorectal tumors, either by colonoscopic biopsy or surgery. The tumor is immediately fixated by using 10% formaldehyde buffering solution and made into paraffin blocks or formalin-fixed and paraffin-embedded tissues (FFPET). It is used to determine the histology of the tumor and to examine biomarker test. Immunohistochemistry test (IHC) is a special staining process performed on cancer tissue. IHC is used for the detection of chromosome instability (CIN), DNA-MMR defect (MSI status), and KRAS mutation status. PCR test is used for the detection of microsatellite Instability (MSI) and CpG Island Methylation Phenotype (CIMP).[3,7,9]

Laboratory Examination

Complete blood count, urinalysis, serum chemistries e.g., hepatic function panel, kidney function tests, etc. CEA tests is recommended before surgical operation and follow up after operation if there is a suspicion of cancer recurrence. An increase in the concentration of CEA after operation suggested of recurrence.

CT Scan or Magnetic Resonance Imaging (MRI)

If local or systemic metastasis CRC is suspected, the following radiologic studies may be obtained, such as CT scanning of the chest, abdomen and pelvis, MRI or trans rectal USG (TRUS) for rectal cancer. Diagnostic evaluation is conducted for localized staging of rectal cancer with TRUS: 80-95% accuracy of distinction between T1/2 vs T3 tumors. MRI is high degree of accuracy for prediction of circumferential resection margin with less operator dependent, and it allows for a study of stenotic tumors and pelvic adenopathy.[7,16]

BIOMARKERS IN CRC

As targeted therapies continue to emerge in the treatment of mCRC, knowledge and implementation of predictive and prognostic biomarkers will become increasingly important to ensure the best outcomes and to limit toxicity. Biomarker tests may impact selection of the most appropriate treatment strategy.[3,4,7,17-19] The application of novel molecular diagnostic test may lead to an improved survival of CRC patients.[20] The novel markers in conjunction with clinical and pathologic features have allowed for more individualized approaches to patients with CRC.[21]

The location, the stage of the tumor, and the result of biomarker tests can be used for

the assessment of the therapeutic approach. Some cancer treatments, including chemo therapies and targeted therapies, may only work for patient whose CRC have certain biomarkers.[3,4,17,18] Several biomarkers for mCRC include extended RAS (KRAS and NRAS) exons 2,3, and 4 mutations, BRAF V600E mutation, mismatch repair (MMR) or MSI and HER2 (human EGFR2) amplification. In addition, actionable gene fusions such as NTRK fusions or rearrangements (Neurotrophic Tropomyosin Receptor Kinases) are extremely rare but might represent a new target to improve outcomes in the setting. However, recent NGS (next-generation sequencing) approaches can detect all required types of genomic alterations, including amplification, fusions, and MSI.[18] MSI is a key biomarker in CRC, with crucial diagnostic, prognostic, and predictive implications.[22]

It is important for understanding the onset and the progression of CRC that can aid in the early detection of molecular markers and risks to improve the clinical care of CRC patients.[23] Gene mutations have the potential of disrupting several epigenetic patterns (DNA methylation, histone modification, and nucleosome positioning), and those epigenetic modifications can drive genome instability and mutagenesis (a crosstalk). Similarly, epigenetic inactivation of DNA MMR is often associated with genome instability and increased frequency of point mutations of cancer-related genes.[24]

The genes that are most commonly mutated in CRC patients include APC (about 80-82% of cases), TP53 (48-59%), KRAS (40-50%), and PIK3CA (14-18%). Some biomarkers are based on the mutational status of genes (KRAS, NRAS, BRAF) or associated with defects in the DNA mismatch repair system (dMMR). These defects are the underlying mechanism through which MSI status is determined.[17]

KRAS, BRAF BIOMARKER

Mutations in KRAS/NRAS exons 2 (codon 12 and 13), exon 3 (codon 59 and 61), and exon 4 (codons 117 and 146) are predicted due to lack of benefit for anti-EGFR mAb (such as cetuximab and panitumumab) treatments in mCRC. Patients with *RAS-Wild* Tumor who are left-sided demonstrated excellent outcomes with anti-EGFR-based therapy. Patients with BRAFV600E mutations demonstrated a markedly worse prognosis than non-BRAF V600E patients, and a number of sub analyses of mutation appear to suggest benefit from aggressive triplet-based therapy as a frontline therapy. Patients with BRAF V600E demonstrated a benefit in median progression free survival in the SWOG randomized clinical trial of vemurafenib/irinotecan/cetuximab in the second-line setting in comparison with irinotecan/cetuximab.[18,25] The BRAF gene is activated by mutation in 10-12% of all CRC and most frequently occur in codon 600 (BRAF V600).[17,25]

APC

Mutations in the APC gene occur in over 70% of sporadic CRCs, and they are the cause of the FAP cancer predisposition syndrome. The APC protein negatively regulates WNT signaling by facilitating the targeting of the transcription factor B-catenin for ubiquitin-mediated proteasomal degradation.[19] APC mutation in advanced state CRC had poorer overall survival. It can be used to predict the clinical outcome of CRC.[24]

MSI STATUS AND DMMR

Microsatellite Instability (MSI) status results from a deficient DNA mismatch Repair (dMMR) system commonly caused by the inactivation of the MMR genes (MLH1, MSH2, MSH6, PMS2, PMS1, and MLH3). MSI-high (MSI-H) is characterized by instability of two or more loci. MSI status can be determined by two distinct methods, immunohistochemistry analyses (IHC) or PCR based on five markers panel.[4,7,17,24]

MSI-H tumors can be observed in approximately 15% of all CRC patients. Of the 15%, 12% are associated with sporadic CRC, due to sporadic hypermethylation of the promoter of the MLH1 gene. The other 3% of MSI tumors are associated with Lynch syndrome, an inherited cancer syndrome associated with a genetic predisposition to CRC.[17] MSI/MMR testing should be performed in all patients diagnosed with CRC, and may be predicted with immunotherapy in the metastatic setting.[17,18,24]

CRC stage II MSI-H patients have a better prognosis and no beneficial effect of 5-FU.[22] This prognostic significance indicates the need to implement MSI screening for all resected stage II CRC patients. MSI status is less informative in stage III patients.[17] Emerging data suggest that tumors with dMMR or MSI-H respond better to immune check point inhibitors (ICIs).[17] The US FDA approved pembrolizumab, a monoclonal anti-PD1 antibody, for patients with MSI-H CRC. Additionally, Nivolumab and Iplimumab are approved options for refractory stage IV MSI-H patients, after prior therapy with fluoropyrimidine, oxaliplatin, and irinotecan-based therapy.[17,18] MSI is a key biomarker in CRC, with crucial diagnostic, prognostic, and predictive implications. MSI-H status is associated with a better prognosis in early-stage CRC and a lack of benefit from adjuvant treatment with 5-fluorouracil (5FU) in stage II disease. MSI has emerged as a predictor of sensitivity to immunotherapy-base treatment.[22] Pemrolizumab is only effective in mCRC patients with MSI-H status.[25]

CIMP

Epigenetic instability in CRC is manifested as both hypermethylation of gene promoters that contain CpG island and global DNA hypomethylation. Aberrant DNA methylation is present in essentially all CRCs; however, there is a subset of CRCs (approximately 20%) that has an extremely high proportion of aberrantly methylated CpG loci. This class of CRCs has been characterized as having a CIMP. The discovery and classification of CIMP tumors have advanced our understanding of the molecular pathology of CRC, but it has not yet impacted clinical care.[19] CIMP status seems to overlap with BRAF mutations and MMR status. Thus, the independent prognostic value of CIMP needs to be validated.[17]

TP53

TP53 is the most frequent somatic gene mutation, and its mutational status has been associated with a positive response to adjuvant 5-FU therapy in stage III CRC patients. Further studies are necessary in order to determine the role of TP53 as a potential prognostic and predictive biomarker in CRC.[17] Mutations in the TP53 occur in about half of all CRCs, and these mutations promote the malignant transformation of adenoma. Like APC, TP53 is a key tumor suppressor gene that has been extensively studied in CRC, but it currently has no predictive or prognostic role in the clinical setting.[19] TP53 mutations or loss of function are reported in 50-75% of CRC cases.[23]

PI3K

Molecular lesions in the PI3K pathway, which in CRC are primarily mutations in *PIK3CA* and loss of PTEN protein expression. Mutations of genes in this pathway may have the potential to be used as predictive biomarkers for therapies that target the PI3K pathway, mammalian target of rapamycin (mTORC) pathway, as well as the MAPK pathway. Mutations in PI3K pathway genes are observed in up to 40% CRC patients.[19] It is difficult to evaluate the importance of PIK3CA as an independent pre dictive marker as PIK3CA mutations often co-occur with RAS or BRAF mutations.[17]

HER 2 ABERRATION

Human epidermal growth factor receptor 2 or HER2 (Errb2) is a transmembrane receptor of the EGFR family and its activation leads to cell proliferation and apoptosis inhibition. The frequency of HER2 overexpression is reported to be around 5% with ERRB2 amplifications reported in 5.5% of mCRC.[17,25] The implementation of HER2 assessment in daily practice might provide useful information for guiding therapy decision.[17] Several studies suggest the amplifications of ERBB2 negatively predict efficiency and are associated with development of resistance to anti-EGFR therapy.[17,18]

CONSENSUS MOLECULAR SUBTYPES

In late 2016, a large consortium of groups working on CRC combined their efforts and identified four molecular subtypes based on multi gene arrays, which were conserved across all examined studies. These subtypes are referred to as CMS1 (MSI-immune subgroup representing

14% of CRC cases), CMS2 (canonical subgroup accounting for 37% cases), CMS3 (metabolic representing 13% of CRC patients) and CMS4 (mesenchymal representing 23% of CRC cases). CMS subtypes primarily show an association with clinical outcomes. The clinical impact of the identification of these subtypes remains relatively limited.[17,23,25]

MicroRNAs

MicroRNA (miRNAs) are considered to be exceptional biomarkers due to their involvement in multiple physiologic pathways and their stability in paraffin-embedded tissue (FFPET), which is an important factor for the translation of biomarkers into the clinics. miRNAs play an important role in the regulation of intracellular processes via the post-transcriptional regulation of gene expression.[17] Several specific miRNAs were identified as predictive or prognostic biomarkers in CRC.[20, 25] Numerous miRNAS have been reported in tumor specimens from CRC patients, and several representative miRNAs have been identified in tumor tissues that have prognostic value and response to anti-cancer drugs in CRC patients.[25,26] Despite the numerous studies of miRNAs and extensive analyses of their expression, the role and function of many individual miRNAs in CRC remains poorly understood.[27]

TUMOR LOCATIONS

The most interesting concept in CRC is the impact of the primary tumor location. Colon is known to have two distinct embryological origins, namely the midgut for the proximal colon and the hindgut endoderm for the distal colon. Additionally, the two parts of the colon have different blood supplies, distinct microbiome populations and are associated with different biological features. Proximal colon cancer shows a worse prognosis than distal colon cancer. Anti-EGFR therapy should be limited to distal colon cancer with KRAS wild type.[17] Primary tumor location affects response to targeted therapy and is essential for the treatment selection for RAS wild type mCRC.[18]

A study on molecular biomarker in CRC based on three locations (proximal colon, distal colon and rectum) was published (Effendi et al., 2013). In this study, tumor tissues of CRC patients from three locations were examined by IHC and PCR test. The protein expressions were determined by IHC for APC, dMMR (MLH1, MSH2, MSH6, and PMS2), while microsatellite instability-high (MSI-H) by PCR was based on 5 markers of BAT25, BAT26, D2S123, D5S346, D17S250, known as Bethesda panel. MSI-H was considered if there were ≥ 2 of abnormal markers.[4,7,24] There were differences in the characteristics of CIN, MMR, and MSI-H found in colorectal cancer patients based on different locations. The MLH1 protein expression negative was prominent in proximal colon cancer, MSH6 in distal and rectal cancer, and APC in distal colon cancer respectively. The proportion of MSI-H displayed a tendency to occur in proximal rather than in distal colon or rectal cancer. Nevertheless, these findings suggest that the underlying carcinogenic pathway or molecular backgrounds differ according to the cancer locations among CRC patients in this region. The CRC in each location has its specific molecular characteristics.[3,4,7]

Right colon cancer or proximal colon cancer had better outcome at stage II in comparison with Left CC, and it had better outcomes at stage I and II in comparison with rectal cancer. In other words, Right CC was associated with a relatively favourable outcome in an early-stage disease, but had an opposite outcome in regional and metastatic disease. In this case, tumor heterogeneity was considered as the main potential reason. Tumor phenotypes may vary depending on the process of tumor infiltration and metastasis.[14]

Cancer Stem Cells (CSCs)

CSCs have been put forward to be one of the determining factors that contribute to intra-tumor heterogeneity and the potential implications for CRC therapy.[27] CSCs can escape the chemotherapy and differentiate into mature cancer cells, resulting into cancer recurrence and metastasis. Therefore, development of therapy targeting CSCs has a¬therapeutic potential, and it is important to identify approaches in combination with conventional therapy for

targeting and eradicating CSCs.[3,4,9,28-30]

TREATMENT

Most CRC patients are treated with surgery to remove the tumor tissue, and some of the CRC patients recurred. Chemotherapy used as adjuvant or neoadjuvant therapy also presents several problems, in which these treatments are useless in tumor cells with chemo-resistance. Despite advanced treatment strategies, CRC is rarely cured completely due to recurrence. Chemotherapy can only shrink tumors by killing the active tumor cells but miss the quiescent colorectal cancer stem cells (CSCs). This leads to resistance and relapse, and usually includes systemic and local toxicity of chemotherapy. CSCs are considered as the origin of tumorigenesis, development, metastasis and recurrence in theory.[3,8,9]

Surgery is the primary treatment for CRC, that it is limited to the colon or rectum. It aims to remove cancerous tissue of an early stage cancer, including tumors and nearby lymph nodes, preventing the cancer from spreading. In the later stages**,** surgery cannot stop the cancer from spreading.

Systemic therapies for localized and advanced CRC include chemotherapy, targeted therapy, immune therapy, and a newly developed therapy. Systemic therapy for CRC aims to downsize the primary tumor or metastases in order to convert them to a resectable status and increase progression-free survival.

Chemotherapy drugs destroy cancerous cells throughout the body. It may shrink a tumor before surgery. It can also help relieve symptoms in the later stages. Chemotherapy can have widespread adverse effects, as it targets both cancerous and healthy cells.

Targeted therapy involves taking drugs that target specific protein to slow or prevent the growth of cancer cells. The adverse effects are usually less severe than those of chemotherapy because these drugs only target specific cells.

Immunotherapy can have possible adverse effects include an autoimmune reaction, in which the body mistakenly attacks its own cells. If CRC spreads to organ beyond the colorectal, progressing to stage 4, it is not possible to cure it.

Recently numerous *newly developed therapies* are available. Other option for stage 4 CRC may include: surgery to remove a blockage, radiation therapy or chemotherapy to reduce the size of tumors, pain relief, treatment for side effects of medication, and counseling. Therapeutic management should involve a multidisciplinary approach that includes diagnostic accuracy, surgical technique (high quality surgery), biomarker test, optimal selection of drug treatment or procedure and informed consent.[16,31-33]

Colon cancer

a. Stage I: Surgery
b. Stage II-III: Surgery ± adjuvant chemotherapy
c. Stage IV: Chemotherapy ± palliative surgery.

Rectal cancer

a. Stage I: Surgery (local excision) or total mesorectal excision (TME).
b. Stage II-III: Neoadjuvant chemoradiation followed by resection, followed by adjuvant chemotherapy.
c. Stage IV: Chemotherapy ± surgery.

Surgery is also performed if there is a perforation or blockage due to a tumor.

Sometimes endoscopic stent placement is required before surgery for partial obstruction due to CRC.[16, 31-33]

Chemotherapy for advanced CRC and metastasis:[16]

a. 5-FU/Leucoferin+ Oxaliplatin (Folfox),
b. 5-FU/LV+ irinotecan (Folfiri), or
c. Capecitabin + Oxaliplatin (CapeOx).

TARGETED THERAPY

Targeted therapies are recommended for patients with KRAS/NRAS/BRAF mutated tumors, or wild-type tumors, HER2-amplified tumors and NTRK gene fusion-positive tumors. Development and approval of novel targeted therapies such as monoclonal antibodies against EGFR and VEGF have significantly increased the median survival of CRC patients with advanced or metastatic disease.

Monoclonal antibodies against EGFR (cetuximab or panitumumab) are recommended for patients with KRAS/NRAS/BRAF wild-

type tumors. There are no roles for anti EGFR therapy in KRAS mutation CRC, as well as for dual anti EGFR and anti VEGF combination therapy.[16, 31,33-36]

Targeted cytotoxic drugs against colorectal CSCs marker research will provide more effective therapeutic results as well as reduce resistance and recurrence.[3,37,38]

CR-CSCS are defined with a group of cell-surface markers, such as CD44, CD133, CD24, epithelial cell adhesion molecule (EpCAM), LGR5, and acetaldehyde dehydrogenase (ALDH). They are highly tumorigenic, aggressive, radioresistant and chemoresistance and thus are critical in the metastasis and recurrence of CRC. Therefore, targeting CR-CSCs may become an important research direction for the future cure of CRC.[39,40] The biological activities of CSCs are regulated by several pluripotent transcription factors, such as OCT4, Sox2, Nanog, KLF4, and MYC. In addition, many intracellular signaling pathways, such as Wnt, NF-kB(nuclear factor-kB), Notch, Hedgehog, JAK-STAT (Janus kinase/signal transducers and activators of transcription), PI3K/AKT/mTOR (phosphoinositide 3-kinase/AKT/mammalian target of rapamycin), TGF(transforming growth factor)/ SMAD, and PPAR (peroxisome proliferator-activated receptor), as well as extracellular factors, such as vascular niches, hypoxia, tumor-associated macrophages (TAM), cancer-associated fibroblasts, cancer-associated mesen chymal stem cells, extracellular matrix, and exosome, have been shown to be very important regulators of CSCs.[41]

Tumor microenvironment (TME), contains both cellular components with cancerous and non-cancerous cells such as stromal myofibroblast, endothelial cells, immune cells and cancer-associated fibroblast (CAFs), and non-cellular components including extracellular matrix (ECM), cytokines, growth factors and extracellular vesicles. In the tumor stroma, CAFs secrete the cytokines CXCL1 and CXCL2 as well as the interleukin-6, which promote angiogenesis and tumor progression.[42,43] CSCs reside in anatomically specialized regions of the TME, known as CSC niche, which retain their properties and protect them from anticancer drugs, contributing to their enhanced resistance to treatment. TME and metabolic plasticity may also be involved in therapeutic failure by imposing selective pressures on CSCs that lead to chemoresistance and cancer progression. Therefore, the development of new therapies targeting CSCs taking into account the TME and tumor metabolism, represents an interesting approach to overcome resistance to therapies.[42, 43]

CSCs are thought to be responsible for tumor initiation and dissemination, drug and radiation resistance, invasive growth, metastasis, and tumor relapse. Specific pheno types could be used to distinguish CSCs from non-CSCs. CSCs are modified by the aberrant expression of several microRNAs.[44] Thus, it is very difficult to target CR-CSCs. Targeting cytotoxic drugs to CR-CSCs with the help of stem cell surface markers provides a useful method to treat CRC. Also, the use of inhibitors targeting drug-detoxifying enzymes, drug-efflux pumps, or transcription factors of CSCs represents a novel approach to target the CSCs and reduces cancer recurrence and metastasis.[7, 45]

Immunotherapy

Immune checkpoint inhibitors (ICIs) are a type of immunotherapy often made from antibodies. ICIs target co-inhibitory receptors, such as CTLA-4 and programmed cell death protein 1 (PD-1) on T cells and other immune cell sub populations, or their ligands, such as PD1 ligand 1 (PD-L1) on tumor cells and various immune cells. In CRC, it has been shown that only patients with the subset of dMMR or MSI-H tumors are likely to respond to treatment with ICIs.[46-48]

Therapeutic approaches for the treatment of CRC include surgery (surgical resection), local therapies for metastatic disease, a systemic therapy comprising chemotherapy, targeted therapy and immunotherapy, and palliative chemotherapy.[42]

Surgery can be associated with neoadjuvant therapy in order to shrink tumor mass and fascilitate medical operation and/or with adjuvant therapy to limit cancer recurrence. Importantly, neoadjuvant chemotherapy, possibly coupled with radiotherapy, is mainly indicated for rectal cancers.[42.] Treatment options and

recommendations depend on several factors. These factors‒include: the patient's overall health, possible side effects, the type, the size and the stage of the tumor, the location of the tumor, and its mutational and MMR status, or biomarkers.[42]

The lines of treatment in mCRC currently involve a combination of targeted therapies that inhibit the EGFR (cetuximab and panitumumab), or angiogenesis (bevacizumab, regorafenib, aflibercept, or ramucirumab), together with chemotherapy (5-fluorouracil, irinotecan, oxaliplatin, Folvox, Folfiri, or CapeOx).[46]

Systemic therapies for localized and advanced colorectal cancer[42]

1. Chemotherapy:
   1a 5-Fluorouracil (antimetabolite).
   1b. Capecitabine (antimetabolite).
   1c Irinotecan (Topoisomerase inhibitor).
   1d. Oxaliplatin (Alkylating agent).
   1e Trifluridine/Tipiracil (Nucleoside analog / thymidine phosphorylase inhibitor)
   [1a to 1d: Recommendations for: Localized and advanced tumors].
2. Targeted therapy:
   2a. Bevacizumab (mAb anti-VEGF-A).
   2b. Regorafenib (Multikinase inhibitor targeting e.g. VEGFR and BRAF).
   2c. Aflibercept. (Recombinant fusion protein blocking VEGF -A/B).
   2d. Ramucirumab (mAb anti-VEGFR-2.
   [*2a to 2d: Recommendations for: KRAS/ NRAS/BRAF Mutated tumors*].
   2e. Cetuximab (mAb anti-EGFR).
   2f. Panitumumab (mAb anti-EGFR).
   [2e to 2f: Recommendations for: KRAS/ NRAS/BRAF Wild-type tumors]
3. Immunotherapy:
   3a. Pembrolizumab (mAb anti-PD-1).
   3b. Nivolumab (mAb anti-PD-1)
   3c. Iplimumab (mAb anti- CTLA-4).
   [3a to 3c: Recommendations for: MSI-High tumors].
4. Newly developed therapy.
   4a. Vemurafenib (BRAF inhibitors).
   4b. Dabrafenib. (BRAF inhibitors).
   4c. Encorafenib. (BRAF inhibitors).
   4d. Trametinib (MEK inhibitors).
   4e. Binimetinib (MEK inhibitors).
   [*4a to 4e: Recommendations for: BRAF V600E mutated tumors*].
   4f. Trastuzumab. (mAb anti HER2).
   4g. Pertuzumab. (mAb anti HER2).
   4h. Lapatinib. (Dual HER2/EGFR inhibitor)
   [*4f to 4h: Recommendations for: HER2 amplified tumors*].
   4i. Larotrectinib. (TRK inhibitors)
   4j. Entrectinib. (TRK inhibitors).
   [*4i to 4j: Recommendations for: NTRK gene fusion-positive tumors*].

* monoclonal antibody anti-programmed cell death receptor-1 (mAb anti-PD-1)
* anti -cytotoxic T lymphocyte associated antigen -4 (anti- CTLA-4)
* TRK- tropomyosin receptor kinases.
* MEK-Mitogen-activated kinases.
* NTRK- neurotrophic tropomyosin kinase.

CONCLUSION

Clinical signs and symptoms of CRC vary, and they are related to the location of cancer whether in proximal, distal colon or rectum, the tumor size, and the stage of CRC. Colonoscopy is the gold standard diagnostic tools, and to prevent CRC by screening and removing premalignant lesions. Biomarkers will give the information on prognostic value, serving as predictive marker for the possibility of response to chemotherapy, biological target agent, immunotherapy, newly developed therapy or targeted therapy. The approved target therapy related to the markers can be used for the selection of the treatment. The more precisely targeted therapies which can selectively target CSCs but spare normal stem cells are necessary and further research on the Colorectal-CSCs markers is greatly needed. The role of the CSCs surface markers as well as targeted therapies to CR-CSCs are currently being studied by various research centers.

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