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A Review: Colorectal Cancer and Its Various Biomarkers

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ABSTRACT

Colon cancer is the second most common cause of cancer-related deaths worldwide. It was anticipated that there were over 1.9 million new instances of colorectal cancer and over 930,000 deaths from the disease in 2020. There were significant regional differences in occurrence and mortality rates. The annual burden of colorectal cancer is expected to rise to 3.2 million new cases (a 63% increase) and 1.6 million deaths (a 73% increase) by 2040.

The two primary causes of colorectal cancer patients' poor prognosis are tumor recurrence and treatment resistance. Numerous solid tumors, including CRC, have been found to include cancer stem cells (CSCs).

Tumor recurrence is mostly caused by cancer stem cells (CSC), which are also resistant to chemotherapeutic medicines and cannot be eliminated after chemotherapy. Biomarkers that are strongly associated with the morbidity of the disease may be useful for prognostic or diagnostic biomarkers.

In this review, we feature many studies that show biochemical, molecular, and immunological biomarkers for colorectal cancer (CRC) which play a main role as a diagnostic and prognostic tool.

1. Colorectal cancer

Colorectal cancer (CRC) is heterogeneous and causes malignant tumors, or polyps, to grow in the inner walls of the colon and rectum [1]. Colorectal cancer (CRC) is the world's third most common and second deadliest cancer, accounting for 10.2% of new cases and 9.2% of cancer-related deaths [2]. Overall survival (OS) at 5 years from initial diagnosis ranges from 87-90% in stage I-II to 68-72% in stage III and drops to 11-14% in stage IV metastatic CRC (mCRC) [3].

1.1. Signs and symptoms of Colorectal cancer

Colorectal cancer symptoms vary depending on where the tumor is located in the intestine and whether it has migrated to other parts of the body (metastasis). The typical warning indications include

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worsening constipation, blood in the stool, a decrease in stool diameter (thickness), loss of appetite, weight loss, and nausea or vomiting in people over the age of 50. High-risk symptoms in people over the age of 50 include rectal bleeding and anemia. Weight loss and changes in a person's bowel habits are usually only considered if they are linked with rectal bleeding [4].

1.2.Causes of Colorectal cancer

There is a positive correlation between the age of those who acquire colorectal cancer and the risk. Different lifestyles across the globe help to highlight the fact that changing one's lifestyle can genuinely impact the frequency of colorectal cancer [5, 6].

Other risk factors for colorectal cancer include smoking, drinking too much alcohol, eating a lot of red and processed meats, having inflammatory bowel disease, being obese, having diabetes, and having a family history of colorectal cancer. Emerging data suggests that the risk of colorectal cancer may also be elevated by infection with Helicobacter pylori, Fusobacterium spp., and other putative infectious pathogens [7, 8].

1.3.Staging of Colorectal cancer

Colon cancer is staged using the American Joint Committee on Cancer (AJCC) tumor/node/metastasis (TNM) classification and staging system. The TNM (T, tumor size and any tissue metastasis; N, lymph node metastasis; and M, metastasis) helps patients with cancer, including those with colorectal cancer to give prognostic information and to make educated decisions. Nonetheless, research has demonstrated significant differences in the clinical outcomes of patients with colon cancer at the same TNM stage [9].

Currently, TNM classification is determined by anatomical assessment. However, more prognostic and/or predictive markers are needed for accurate prediction. Finding out if early Treatment Success Rate (TSR) evaluation and early therapy stratification can improve survival in particular individuals is crucial. There have been suggestions for additional biomarkers based on characteristics of tumor cells, such as morphology, molecular pathways, genetic changes, cell of origin and gene expression, and immune response of tumor cells. Their drawback, though, is that the cost of genetic and transcriptome data is much more than that of traditional pathological examination by microscopy, which is dependable, rapid, and low-cost. Thus, it is preferable to have a disease biomarker that is easy to evaluate [10].

1.4.Screening options, diagnosis, and prognosis of colorectal cancer

About 90% of people with colorectal cancer survive five years if the disease is detected early. This percentage drops if the cancer has spread outside of the colon or rectum. For this reason, early detection is crucial to prolonging the survival of individuals with colorectal cancer. Nowadays, stool-based testing and imaging are the main ways to screen for colorectal cancer. Colonoscopy, stool-based testing, Cologuard (stool DNA), flexible sigmoidoscopy, and computed tomographic colonography are the five categories into which imaging, and stool-based tests may be further separated. The

benefits and risks associated with each test vary (Figure. 1) [11].On the other hand, the prognosis is improved for such cells when molecular analysis, typically with reverse transcriptase polymerase chain reaction (RT-PCR) [12, 13]. Molecular tumor-cell detection in lymph nodes (LNs) by RT-PCR was linked to poor overall survival, disease-specific survival, and disease-free survival, according to a comprehensive evaluation of 39 studies [12]. One of the most helpful techniques for determining the expression of biomarker mRNA is real-time quantitative RT-PCR analysis. Technology has many advantages over histology, including the ability to evaluate huge tissue volumes, up to the entire lymph nodes which can be analyzed [14].

1.5.Biomarkers of Colorectal Cancer and Cancer stem Cells

Biomarkers are a trait that is objectively tested as a sign of normal biological processes, pathogenic processes, or pharmacological reactions to a therapeutic intervention. The Biomarkers Definitions Working Group of the National Institutes of Health has delineated the fundamental applications of biomarkers in medicine, encompassing, but not restricted to, the diagnosis of illness or condition, the tracking of illness or condition progression, the evaluation of treatment or pharmacodynamic response, the prognostication or risk-stratification of patient outcomes, and the prediction of patient subgroups that would exhibit differential responses to interventions [15].Biomarkers can be detected in solid tumor tissue, in a lymph node, bone marrow, peripheral blood, or other biological materials (urine, ascites, and stool) [16]. As a diagnostic and prognostic tool for colorectal cancer (CRC), biomarkers can be classified as biochemical, molecular, and immunological biomarkers.

1.5.1. Biochemical biomarkers

1.5.1.1.Carcinoembryonic antigen (CEA)

CEA is a member of the immunoglobulin family known as CEA-related cell adhesion molecules (CEACaMs). The activities of endothelial cells, such as adhesion, proliferation, and migration of cells in-vivo and in-vitro, are directly linked to CEA [17], It is believed to prevent apoptosis and thus be implicated in the etiology of tumors. It is found on the endoluminal side of the cell membrane of normal cells. While gastrointestinal tumors are the primary association of CEA, research indicates a strong link between CEA and thyroid, breast, lung, ovarian, and mucinous adenocarcinomas of the cervix malignancies. For patients with colorectal cancer who had adjuvant chemotherapy and surgical resection, CEA is a highly predictive biomarker, [18].

A poor prognosis is linked to an elevated CEA level of more than 5 μg/L at the time of a new colorectal cancer diagnosis [19]. Normalization of high CEA levels following surgery, however, is not linked to a dismal prognosis. Therefore, regular CEA evaluation before surgical therapy is not recommended, and post-operative detection is typically more helpful in prognosticating and identifying recurrence within the first year following surgery.

The follow-up after colorectal surgery (FACS) experiment demonstrated that CEA level

monitoring in patients with colorectal malignancies after initial treatment was useful in identifying cancer recurrences that may be treated with curative intent [20]. The national recommendations for colorectal cancer in North America and Europe also support measuring CEA during post-operative follow-up [21,22].

In order to evaluate the response to resection and systemic therapy (chemotherapy/radiotherapy) in colorectal cancer, serial CEA testing is advised before to the start of treatment and then every three months during active treatment and active surveillance [23].

According to other studies, RT-qPCR of CEA mRNA which is a sensitive way to identify tumor cells in the lymph nodes of patients with colorectal cancer when combined with MUC2 mRNA improves the ability to predict clinical prognosis. Additionally, CEA had the greatest expression level per colon cancer cell, the highest tissue specificity (specificity index 35,200), and the least amount of variance in expression levels between and within primary CRC tumors. As a result, if CEA mRNA is used alone, it is the preferred marker [24].

1.5.1.2. Carbohydrate antigen (CA 19.9)

CA 19.9 is a glycoprotein with a large molecular weight that may be released into the bloodstream. The diagnosis of stomach, colorectal, and pancreatic cancers is done using this marker. Similar to CEA, it is not restricted to a certain histological type of cancer or organ of origin. Compared to CEA, CA 19.9 is less sensitive [25]. The tests of CA 19.9 and CEA combined may improve diagnostic sensitivity in the diagnosis of colorectal cancer. Furthermore, the assessment of the disease's stage and survival rate uses the measurement of both markers as a postoperative prognostic factor. The greater the disease stage, the higher the CA 19.9 concentration and sensitivity; however, they are not correlated with the location of the tumor or the number of positive lymph nodes [26].

1.5.2. Molecular biomarkers

For a while now, researchers have been interested in the potential applications of molecular prognostic biomarkers to predict the course of illness and likelihood of survival [27].

1.5.2.1.RAS Family:

Mutations in RAS family genes such as KRAS, NRAS, and HRAS play critical roles in several tumor types, including CRCs, according to strong scientific evidence. The majority of CRCs exhibit hotspot mutations in the Kirsten RAS gene, with about one-third of CRCs exhibiting these alterations [28]. These mutations are frequently linked to carcinomas of the colon on the right side, and their expression gradually diminishes as one moves from the colon's proximal to distal parts [28]. While there has been much discussion in recent decades on the prognostic relevance of KRAS mutations in primary and metastatic colon cancers, there is conflicting data regarding the effects of KRAS changes on the survival of patients with colorectal cancer [29]. However, in CRC patients, NRAS mutations are less common. Remarkably, it has been shown that CRCs with NRAS mutations had a worse prognostic impact than CRCs with KRAS mutations [30]. On the other hand, mutations of the RAS

gene family are mutually exclusive with mutations of BRAF and other elements of the MAP kinase cascade, and they result in a constitutive activation of the RAS-RAF-MEK-ERK-MAP kinase pathway, which is involved in cell growth, proliferation, and differentiation [28,31].

1.5.2.1.1. KRAS

KRAS is an oncogene that produces small GTPase transducer proteins that bind to guanine triphosphate. KRAS proteins are found on the cell membrane and are also referred to as p21 [32]. During signal transduction, KRAS is temporarily active [33]. This gene is mutated in codons 12 (82–87%) associated with mucinous colorectal cancer (CRC) and 13 (13%–18%) associated with non-mucinous colorectal cancer (CRC), which is more aggressive and has a higher incidence of metastases [34].
Mutations in the KRAS gene cause the signal transduction system to be continuously activated, which transforms and renders anti-EGFR antibody treatment useless [35]. Research has demonstrated that the KRAS mutation functions as a negative predictive marker by targeting anti-EGFR treatment, which has been proven to considerably increase overall survival and progression-free survival for patients with KRAS-WT CRC [36].
According to a different research, patients with KRAS-WT respond better to therapy when cetuximab is administered than when the medication is not given to them [37]. When treated with FOLFOX alone or in combination with cetuximab, patients with mutant KRAS showed comparable outcomes. Thus, it is possible to think of the mutant KRAS as a predictor that points towards the most effective treatment approaches [32].

1.5.2.2.BRAF

The BRAF oncogene is a gene that codes for the BRAF protein, also referred to as serine-threonine kinase. This protein is linked to cell growth and is a regulator of the MAPK pathway [38], making it a potential prognostic biomarker and therapeutic indicator for CRC patients [39]. Codon 600 of the BRAF gene is where CRC-related mutations are most common [40]. Five to nine out of every hundred individuals with colorectal cancer had a mutation in the BRAF gene, which is caused by the conversion of valine to glutamic acid [41].
There is evidence that cancer growth and development are events that occur when there are mutations in KRAS and BRAF [32]. Research has indicated that compared to wild-type BRAF, mutant BRAF exhibits a higher methylation rate. Furthermore, it demonstrated a strong correlation between the BRAF mutation and MSI [33]. Mutated BRAF can affect any portion of the colon and rectum, although it is more common in women and those over 70. It is primarily found in the right colon. Testing for this mutation is advised in stage IV patients in order to more effectively focus therapy [41].

1.5.2.3.TP53

The tumor suppressor gene P53, also known as TP53, encodes a cytoplasmic protein with transient expression that affects the cell cycle, apoptosis, senescence, and DNA repair while functioning as a tumor suppressor. A crucial part in maintaining stability and preventing genomic mutation is played by

TP53. A persistent protein that disrupts the DNA repair pathway is produced when a gene is mutated [42].

While studies have demonstrated that the dosage of these antibodies in peripheral blood is irregular and their sensitivity is less than 30%, the continuous expression of the protein can result in the immune system's identification and the generation of antibodies against TP53 [43]. TP53 mutations are seen in around 60% of colorectal cancers and can lead to a progression from adenoma to CRC carcinoma. Thus, the discovery of this mutation serves as a prognostic marker in CRC patients, indicating a poor prognosis and limited survival [44].

1.5.2.4.Microsatellite instability (MSI)

MSI are small repetitions of DNA sequences found throughout the human genome. Microsatellite instability (MSI) is caused by a DNA mismatch repair system (MMR) defect, namely the inactivation of the four MMR genes (MSH2, MLH1, MSH6, and PMS2), which results in the inability to correct insertion or exclusion of repeat during DNA replication [45]. It is a very changeable phenotype. MSI occurs in around 15% of all colorectal cancers [46].

CRC with microsatellite instability is mucinous, has poor cell differentiation, and has a high lymphocyte infiltration, particularly in the right colon [47]. Surprisingly, those with MSI had a better prognosis than those without it. This allows it to be regarded as a possible prognostic marker for CRC patients, and MSI status may be determined using a polymerase chain reaction (PCR) test with a panel of five specific markers (BAT25, BAT26, D2S123, D5S346 and D17S2720) [48].

1.5.2.5.GAPDH

GAPDH Several substances modulate mRNA levels and influence GAPDH's cancer-related actions (proliferation, tumor development, and chemoresistance) [49].

Although GAPDH is expressed in the majority of cells with enzymatic function, it is frequently utilized as an endogenous control molecule in gene expression research. CRC has a strong connection with the CD26 gene, indicating a significant risk of malignancy [50].

1.5.2.6.APC

Adenomatous Polyposis Coli (APC) is a suppressor gene found in familial adenomatous polyposis (FAP). This epigenetic alteration caused by a mutant APC is responsible for most instances of sporadic CRC, with 70% to 80% of individuals carrying this mutation [51].

APC functions as an antagonist to the gene WNT signaling pathway. APC modulates a variety of cellular processes, including migration, adhesion, transcriptional activation, and apoptosis [52].

The association of the three APC polymorphisms (D1822V, E1317Q, and I1307K) in the development of CRC was evaluated, and it was discovered that carriers of the

E1317Q variant had a low risk of CRC, whereas I1307K showed an increased risk of CRC compared to wild type I1307Q [53].

However, there is no correlation between APC promoter methylation and overall survival in CRC patients [54]. Patients with APC mutations and high miR-21 expression in advanced CRC had a worse overall survival. APC mutation and elevated miR-21 expression can be utilized clinically to predict CRC [52]. Different authors believe that hypermethylated APC is a useful biomarker in the early detection of CRC, as well as a potential therapy target, if customized and targeted to the mutation implicated [51].

1.5.3. Immunological biomarkers

Immunologic biomarkers can be predictive or prognostic, just as other oncology biomarkers.

1.5.3.1.APRIL/TNFSF13

Tumor necrosis factor (TNF) protein APRIL/TNFSF13 is crucial for the growth of B lymphocytes, which are used in immunological response [55].

Under normal physiological settings, immune cells in the bone marrow and peripheral organs express this protein. Different tumor cell types, such as those from breast, stomach, bladder, and ovarian cancers, generate APRIL [56 - 59].

APRIL is overexpressed in CRC tissues, according to a number of studies, and higher expression of APRIL is linked to a worse prognosis for CRC patients [60-62].

1.5.3.2.BAFF

v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) is a member of the TNF superfamily and is mostly generated by myeloid cells. By controlling B cell development, activity, and survival, BAFF contributes to immune function. Prior research has indicated that BAFF contributes to the aggressiveness and development of neoplasms [63, 64].

Furthermore, in response to chemotherapy medications for hematological malignancies, both APRIL and BAFF signaling may promote the viability and proliferation of tumor cells. Fascinatingly, high blood levels of APRIL and BAFF are linked to invasiveness and advanced clinical stages of malignancies such as pancreatic, breast, and chronic lymphocytic leukemia [64, 65]. Not all tumors have the same link between BAFF expression and the advancement of the illness [66].

1.5.4. Stem cells biomarkers in Colorectal cancer

Colorectal cancer stem cells (CR-CSCs) may be the starting cells of colon cancer, supporting colon cancer metastasis, and one of the primary causes of therapy resistance and recurrence. Therapy benefits against colon cancer may be enhanced by the elimination of CR-CSCs. [67] The most popular approach is to sort cancer stem cell biomarker proteins. Prior research has revealed the presence of several biomarkers in CSCs, such as CD133, CD44, ALDH1, EpCAM, LGR, and Msi-1 CSC biomarkers differ according to tumor type [68, 69].

1.5.4.1.CD133

This is a cell surface marker known as the prominin-1 protein, commonly referred to as the Penta span-transmembrane cholesterol-interacting CD133 protein, is a cell surface marker. It is present in a variety of malignant tissues, including bone marrow-derived endothelial progenitor cells and intestine bottom crypt cells. Studies have demonstrated that the glycoprotein may serve as a marker for CRC stem cells. Compared to unsorted CRC cell populations, CD133+-enriched cell populations are more capable of engrafting and initiating solid tumor development in immunodeficient mice [70].

CD133+ cells have the ability to progress from non-dysplastic areas of adenoma-polyp-lesions to non-dysplastic serrated hyperplastic polyps, and ultimately to dysplasia, adenomas, and cancers with the help of CSC. This shows that CD133 expression is more often elevated during the early phases of CRC development, which promotes tumor growth. CSCs may express CD133+ due to epigenetic processes including hypermethylation of the CD133 gene promoter. Higher levels of CD133 are strongly associated with poor prognosis and resistance to 5-FU-based treatment in cancer development [71].

Additionally, CD133+ cell populations exhibit greater resistance to traditional radiation therapy, which accounts for the increased risk of recurrence in colorectal cancer as well as additional side effects of radiotherapy. The fact that CD133 cell populations can also result in tumor growth in animals with weakened immune systems, however, raises questions about the usefulness of CD133.

When colon cancer cells are exposed to high levels of hypoxia or stress, they can alternate between the CD133+SW620 and CD133SW620 subpopulations. The P5 promoter of the CD133 gene is regulated by human embryonic colon and kidney cancer cells binding to one of the two ETS sites. This is what HIF-1 and HIF-2 are in charge of. These results collectively indicate that CD133 plays a critical role in the initiation and progression of cancer, indicating the possibility that it could serve as a predictive biomarker for CRCSC [72].

1.5.4.2. CD44

CD44 is a non-kinase, single-span transmembrane glycoprotein family that is expressed on embryonic stem cells and at varying amounts on other cell types such as connective tissues and bone marrow. As a known molecular marker for cancer stem cells (CSC), CD44 expression is also elevated in subpopulations of cancer cells. Ten of the 19 exons that make up the human CD44 coding sequence are consistent across all isoforms. The 10 constant exons encode CD44 in its standard form, or CD44s. The ten constant exons and any combination of the remaining nine variant exons make up the CD44 variant isoforms (also known as CD44v), which are produced by alternative splicing [73].

Hyaluronic acid (HA), which is widely distributed in the extracellular matrix (ECM) and is expressed by both cancerous and stromal cells, is the primary ligand for CD44.

When HA binds to the CD44 ligand binding domain, it causes conformational changes that enable the binding of cytoskeletal components or adaptor proteins to intracellular domains. These modifications then trigger a variety of signaling cascades that cause cell adhesion, invasion, migration, and proliferation [73].

1.5.4.3.Aldehyde Dehydrogenase

ALDH1 has been found as a CSC marker for various cancers. Two of ALDH1's recognized roles include catalysis and the irreversible oxidation of aldehydes to their corresponding carboxylic acids. ALDH1 expression is increased in individuals with metastatic colon disease, in normal tissues, and in poorly differentiated cancer. ALDH1 was also detected in malignant colonic stem cells [74].

1.5.4.4.Epithelial cell adhesion molecule (EpCAM)

EpCAM is a transmembrane protein that is generated by both normal epithelial cells and epithelial malignancies. EpCAM is involved in cell signaling, differentiation, proliferation, and migration in addition to intercellular adhesion [75]. In some cancer types, EpCAM overexpression is associated with worse survival, whereas in others, it is positively correlated [76].

Only a tiny fraction of cancer cells expressing EpCAM high/CD44+ expression was shown to be able to create xenografts when implanted into immunodeficient mice in cases of colorectal cancer (CRC) [77]. Furthermore, in individuals with colorectal cancer, EpCAM high/CD44+ expression was positively connected with tumor invasion and metastasis [78]. overexpression of the cancer stem cells (CSC) marker EpCAM in regional lymph nodes correlates with poor prognosis in Colorectal cancer patients [79].

1.5.4.5.Leucine-rich repeat-containing G protein-coupled receptors (LGRs)

Leucine-rich repeat-containing G protein-coupled receptors (LGRs) are a subgroup of the seven-transmembrane G protein-coupled superfamily, which regulates a range of physiological processes linked to different disorders. LGR4-6, one of its members, exhibits a high degree of similarity. Numerous investigations have examined the biological roles of LGR4-6 in diverse forms of human cancer. When LGR4–6 binds to R-spondin (RSPO) ligands, which are intimately linked to tumor invasion and progression, it activates the Wnt/β-catenin pathway [80].
overexpression of the CSC marker LGR5 in regional lymph nodes correlates with poor prognosis in CC patients [79].
The G-protein-coupled receptor LGR5, often referred to as GPR49, is expressed by normal stem cells in a variety of organs, including the large and small intestine, where it is restricted to the crypt base columnar cells. LGR5 has been discovered as a CSC marker in colorectal cancer

(CRC), and its overexpression has been linked to distant metastases, lymph node expression, and worse overall and disease-free survival.
overexpression of the CSC marker LGR5 in regional lymph nodes correlates with poor prognosis in CC patients [79]. LGR5's nearest homologs are LGR4 and LGR6. Moreover, it has been shown that LGR4 promotes CC cell invasion and metastasis, and that high expression levels of LGR4 are associated with a poor prognosis in CRC patients [81, 82].
LGR6 mRNA levels in lymph nodes indicate a shorter disease-free survival period and combining LGR6 measurements with the CC prognostic markers CXCL16 and CEA greatly improves predictive efficacy. The most important finding of this study When CC patients relapse after surgery and are skipped by histopathology or CEA and CXCL16, LGR6 can be used as a supplemental biomarker. Although it cannot be used to analyze the primary tumor, LGR6 is helpful as a supplementary biomarker in mRNA analysis of LNs from patients with CC.
Two different situations have prognostic significance for LGR6 mRNA analysis. **1)** If the CC patient has LNs that do not express CEA mRNA (that is, CEA mRNA levels below the cutoff level for LNs of control patients) and **2)** if the CC patient has LNs expressing high amounts of CEA mRNA LGR6 mRNA levels distinguish between individuals with a very poor prognosis and those with a less poor prognosis in the previous situation. LGR6 can identify more patients who are at risk since it expresses it differently than the CSC marker LRG5.
It will be possible to find more patients who would benefit from adjunct therapy by using LGR6 mRNA analysis [83].

1.5.4.6.Musashi-1 (Msi-1)

Musashi-1 is an RNA-binding protein that can compete with the elF4G translation initiation factor found in two messenger RNAs (mRNAs): p21/Waf1 and neural stem cells. These results suggest that Musashi-1 may play a key role in the carcinogenesis of CSCs and the formation of tumors. In CRCS C, Msi-1 is involved in the regulation of Bstemness, which controls Wnt/Notch pathways. However, Msi-1 is exclusive to CRCSCs as a generator of stem cells. As the function of the proteins has only been fully investigated in connection to the nervous system, Msi-1's influence has not yet been determined. All of these findings point to the importance of Msi-1 in the development of CRCSC and call for more study [84].

1.6.Treatment of Colorectal cancer

Depending on the diagnosis and stage of the disease, the first line of therapy for colorectal cancer. is surgical excision of the tumor and any metastases. However, when cancer is detected at an advanced stage with metastases, surgical control becomes difficult, and the best choice for these patients is to reduce the tumor with chemotherapy to stop tumor spread and development. This method might potentially be used as an adjuvant therapy before or after surgery to maximize tumor reduction and stability [85].

In terms of neoadjuvant radiotherapy, this is a method that targets tumor cells with high-energy radiation (x-rays, for example) or particles; it is now utilized to treat rectal cancer (RC), not colon cancer. In fact, it is now standard procedure for treating patients with stage II/III rectal cancer in order to shrink the tumor or kill cancer cells that have disseminated. In patients with stage II/III RC who have not had preoperative irradiation, this treatment can also be utilized after resection to eradicate any leftover cancer cells [86]. When it comes to cytotoxic chemotherapeutic medications, they work by specifically targeting rapidly proliferating cells [87].

The current CRC chemotherapy includes both single-agent therapy, which mostly consists of fluoropyrimidine (5-FU)-based treatment (FOL), and multiple-agent regimens that may include one or more medications, such as capecitabine (CAP), irinotecan (IRI), and oxaliplatin (OX), also; The standard techniques in first-line treatment, considering all of the current chemotherapeutic agents, are FOLFOX (5-FU+OX), FOLFIRI (5-FU+IRI), XELOX or CAPOX (CAP+OX), and CAPIRI (CAP+IRI) combination therapy regimens [85]. By inhibiting thymidylate synthase and incorporating its products into RNA and DNA, the antimetabolite medication 5-FU achieves its anticancer effects [88]. OX is a platinum-based chemotherapeutic medication that suppresses the development and proliferation of cancer cells by damaging their DNA, it is frequently used with leucovorin and 5-FU [89].

Leucovorin and 5-FU are also used in conjunction with IRI (Campto), which inhibits DNA topoisomerase to decrease the development and division of these substances [90]. As an oral prodrug of 5-FU for treatment against colorectal cancer, CAP (Xeloda) was authorized; it was subsequently revised to 5-FU after absorption across the digestive tract [91].

The development of targeted medicines, which may alter certain properties of tumor cells directly, including cell proliferation, differentiation, migration, and even the tumor microenvironment, was made possible by the growing understanding of the characteristics of cancer [92].

In the current treatment of metastatic colorectal cancer, immunotherapy and targeted therapy regimens are becoming a more significant alternative in addition to chemotherapy regimens incorporating 5-FU, OXI, and/or IRI. Chemotherapy in combination with or without biological therapies, such as immunotherapy, panitumumab (Vectibix) or cetuximab (Erbitux), epidermal growth factor receptor (EGFR) inhibitors (bevacizumab (Avastin), or ramucirumab (Cyramza)), or angiogenesis inhibitors, may be taken into consideration. EGFR inhibitors, including cetuximab or panitumumab, are a better option for patients with left-sided tumors that have wild-type BRAF, NRAS, or KRAS genes [93]. Regretfully, KRAS mutations are present in 40% of metastatic colorectal cancers (CRCs); these mutations frequently result in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway and are linked to resistance to anti-EGFR medications [94].

Though neuroblastoma Ras viral oncogene homolog (NRAS) and v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations account for just 4 and 10%, respectively, of all CRC cases, they are also linked to less successful responses to these kinds of treatments [94, 95]. Bevacizumab is a humanized monoclonal antibody that targets vascular endothelial growth factor (VEGF) and is one of the anti-angiogenic medications that has a major role in treating metastatic colorectal cancer (CRC) [96]. Similar to bevacizumab, ramucirumab is a fully humanized immunoglobulin G1 monoclonal antibody that binds to the VEGFR-2 extracellular domain with high affinity, preventing all VEGF ligands from binding to this target. It is also a biologic medication that can be used as an angiogenesis inhibitor in patients with metastatic colorectal cancer [97, 98].

When previous therapies fail to control the disease, this medication is typically used in conjunction with folinic acid/ fluorouracil/ irinotecan regimen (FOLFIRI) to treat metastatic colorectal cancer. Regorafenib (Stivarga) is another biological medication that is now on the market. It functions as a multi-kinase inhibitor by deactivating angiogenic and oncogenic kinases, including VEGF 1-3, fibroblast growth factor receptor 1, EGFR, RAF, and tyrosine-protein kinase, it is intended to treat people with colon cancer that has spread to other organs and is not responding to authorized conventional therapy [97, 99].

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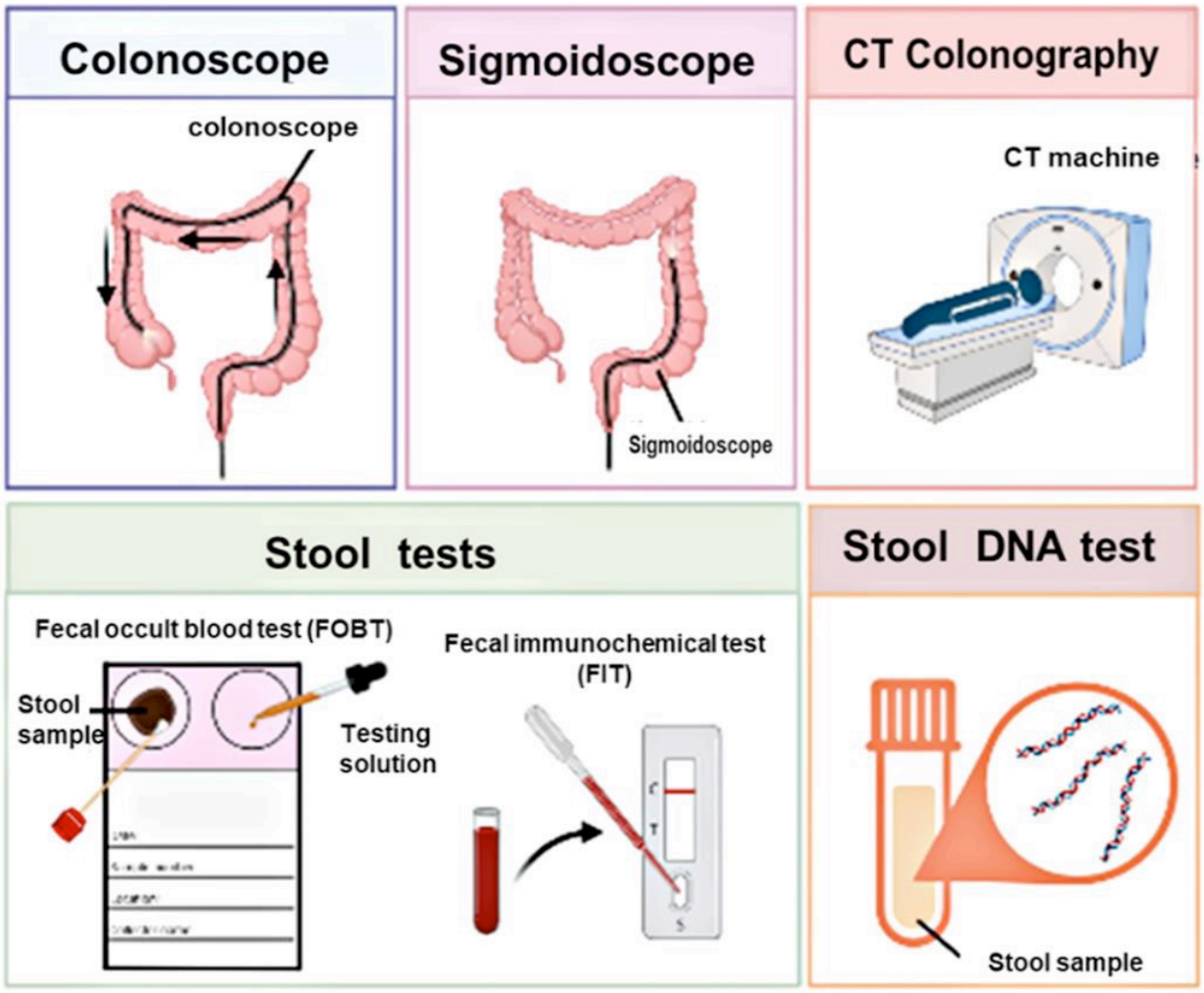

Figure (1): The image of different methods of early screening for CRC [11].