

Systems-level biomarkers identification and drug repositioning in colorectal cancer

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Abstract

Colorectal cancer (CRC) is the most commonly diagnosed fatal cancer in both women and men worldwide. CRC ranked second in mortality and third in incidence in 2020. It is difficult to diagnose CRC at an early stage as there are no clinical symptoms. Despite advances in molecular biology, only a limited number of biomarkers have been translated into routine clinical practice to predict risk, prognosis and response to treatment. In the last decades, systems biology approaches at the omics level have gained importance. Over the years, several biomarkers for CRC have been discovered in terms of disease diagnosis and prognosis. On the other hand, a few drugs are being developed and used in clinics for the treatment of CRC. However, the development of new drugs is very costly and time-consuming as the research and development takes about 10 years and more than \$1 billion. Therefore, drug repositioning (DR) could save time and money by establishing new indications for existing drugs. In this review, we aim to provide an overview of biomarkers for the diagnosis and prognosis of CRC from the systems biology perspective and insights into DR approaches for the prevention or treatment of CRC.

Key Words: Colorectal cancer; Colon cancer; Systems biology; Biomarker; Drug repositioning; Omics

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Core Tip: Colorectal cancer (CRC) is the most commonly diagnosed cancer in women and men worldwide. Due to the lack of clinical symptoms, it is difficult to diagnose CRC in the early stages. There is an urgent need for alternative, inexpensive and easy-to-measure methods for screening and therapy. Systems biology and drug repositioning (DR) approaches are being used to discover biomarkers and novel targets as well as, existing drugs with different indications to develop new therapeutics and treatment

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strategies. Our goal was to provide an overview of systems-level biomarkers and insights into DR strategies for the treatment of CRC.

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INTRODUCTION

Colorectal cancer (CRC) is the most frequently diagnosed cancer type in both women and men worldwide. It occurs in the colon or rectum and affects the large intestine or large bowel. Overall, CRC ranked second in mortality and third in incidence in 2020; and the estimated number of new cases was over 1.9 million with 935000 deaths in 2020[1]. The number is expected to increase to 2.2 million new cases and 1.1 million deaths by 2030[2].

In the absence of clinical symptoms, it is difficult to diagnose CRC in the early stages. According to the American Cancer Society, only 4 of 10 CRC patients are detected in the early stages. If detected in the early stages, the 5-year survival rate can be as high as 90%. The survival rate of CRC patients varies depending on the stages of cancer and metastasis. When metastasis occurs, the 5-year survival rate drops to 14%. Currently, surgical removal is the only curative choice for the treatment of early and localized CRC. In addition, a standard adjuvant strategy for patients with CRC stage III is chemotherapy[3]. As reported by Moertel *et al*[4], the combination of 5-fluorouracil (5-FU) with leucovorin reduced mortality by 33%[4]. In addition, the multicenter international study (MOSAIC), which assessed the adjuvant treatment of CRC with oxaliplatin combined with 5-FU/leucovorin, showed an improvement in patients with stage III CRC[5].

The current various diagnostic strategies for CRC include invasive and non-invasive methods. Invasive methods consist of endoscopy and imaging tests. Endoscopy, which includes sigmoidoscopy and colonoscopy, is the most commonly used method for detecting CRC. Imaging tests such as nuclear magnetic resonance (NMR) and computed tomography (CT) are applied to diagnose severe focal lesions[6]. In addition, positron emission (PET)/CT, particularly fluorine-18-fluorodeoxyglucose PET/CT, is frequently used to diagnose CRC and evaluate patient response to treatment after radiochemotherapy for advanced rectal cancer[7]. As a non-invasive method, a fecal occult blood test is another diagnostic screening for CRC, identifying hemoglobin caused by gastrointestinal bleeding[6].

On the other hand, there is an urgent need for alternative, inexpensive and easy-to-measure methods for screening and therapy. An improvement in technologies in molecular biology provides an opportunity such as prognostic and predictive biomarkers to improve treatment selection or outcome for CRC. Following the completion of the Human Genome Project in 2003, studies of functional genomics (*i.e.* genomics, transcriptomics, proteomics, and metabolomics) have recently increased. Therefore, studies in the field of computational analysis, bioinformatics, and systems biology have gained importance to process data derived from functional genomics and apply a systematic perspective. Consequently, candidate biomarkers made further progress in understanding the mechanism of CRC[8].

The development of new drugs is very costly and time-consuming, as the research and development takes about 10 years and more than \$1 billion[9,10]. Therefore, an approach called "drug repositioning" (DR) could save time and costs by establishing new indications for existing drugs. DR has an effective strategy to provide already clinically tested drugs for complex diseases such as cancer[9].

SYSTEMS BIOLOGY APPROACHES TO DEFINE NEW BIOMARKERS AND DRUG REUSE

Systems biology is an approach that looks at biological systems as a whole and analyzes their interactions and how the interactions affect the behavior and function of the systems[11]. From a holistic view, it allows investigation from the level of cells, tissues, organs, and finally the whole organism. It also combines large amounts of data and reduces it to the levels of genomics, proteomics, and metabolomics to understand the mechanism of complex diseases and build a network model to develop new treatments[12].

Recently, the rising microarray and next-generation sequencing (NGS) technologies lead the accumulation of omics data to identify disease-associated genes, pathways, and biological networks. Therefore, systems biology methods are used to discover biomarkers and novel targets, as well as to develop novel therapeutics and treatment strategies. Genomics studies can enable early diagnosis, post-surgery surveillance, prediction of prognosis, and treatment response through the discovery of single nucleotide polymorphisms, somatic structural variations, copy number alterations, and chromosomal rearrangements in the genome[13]. Transcriptomics studies, involve the analysis of total RNA content, consisting of non-coding RNAs (ncRNAs), microRNAs (miRNA), long ncRNA (lncRNA), circular RNAs, piwi-interacting RNAs, and small nuclear RNAs. Unlike transcriptomics, proteomics provides information on protein function, protein-protein interactions, post-translational modifications, and is therefore important for discovering protein biomarkers in disease. However, proteins rather than DNA or RNA are usually selected as drug targets. The metabolomics approach works on metabolites that are essential for the growth, maintenance, and normal function of cells, such as amino acids, fatty acids, organic acids, sugars, *etc.*[14].

In short, systems biology strategies can unravel the mechanism underlying disease, provide therapeutic alternatives, and biomarkers that have diagnostic, prognostic, or theranostic properties by implementing different omics levels. While difficulties in cancer diagnosis and treatment regularly increase due to confounding pathogenesis and cellular heterogeneity, comprehensive analysis through the systems biology approach consistently helps to gain a comprehensive understanding of disease mechanisms and a greater vision in terms of diagnostic and prognostic biomarkers and targeted drug discovery[15,16]. The integration of high-throughput omics data from different biological levels has been the cornerstone of the systems biology approach of CRC. Moreover, predictions for drug development can be facilitated by the use of systems biology. The identification of biological targets and new promising drugs can be achieved by using *in silico* methods for DR by collecting clinical data at different omics levels and analyzing them within systematic and integrative pipelines[9].

In this review, we aim to provide an overview of systems-level biomarkers such as diagnostics and prognostics (Figure 1) and provide a deep understanding of the DR strategies for the treatment of CRC.

SYSTEMS-LEVEL BIOMARKERS

Despite considerable progress in CRC research aimed at elucidating the molecular mechanisms underlying disease carcinogenesis, the number of biomarkers that are applied in the clinic as routine practice to estimate risk, prognosis, and response to treatment is limited[15]. The lack of reliable and robust biomarkers to screen, monitor, and prevent CRC is a consequence of the heterogeneous nature of this disease and its complex multifactorial pathology[17].

Recent developments in proteomics, transcriptomics, metabolomics, and genomics have increased the number of potential biomarkers, which may ultimately advance the clinical management of CRC to reduce mortality and have led to a better understanding of not only disease progression but also the establishment of molecular biomarkers[8].

Diagnostic markers

A diagnostic biomarker is a measurable indicator that predicts or suggests the presence of a disease or related condition or determines a person with a subtype of the disease[18]. Screening strategies for CRC allow the detection and removal of adenomatous polyps and other premalignant lesions, resulting in a significant reduction in CRC mortality[19]. For these screening strategies in CRC, the identification of early, non-invasive, specific, and robust biomarkers remains an important

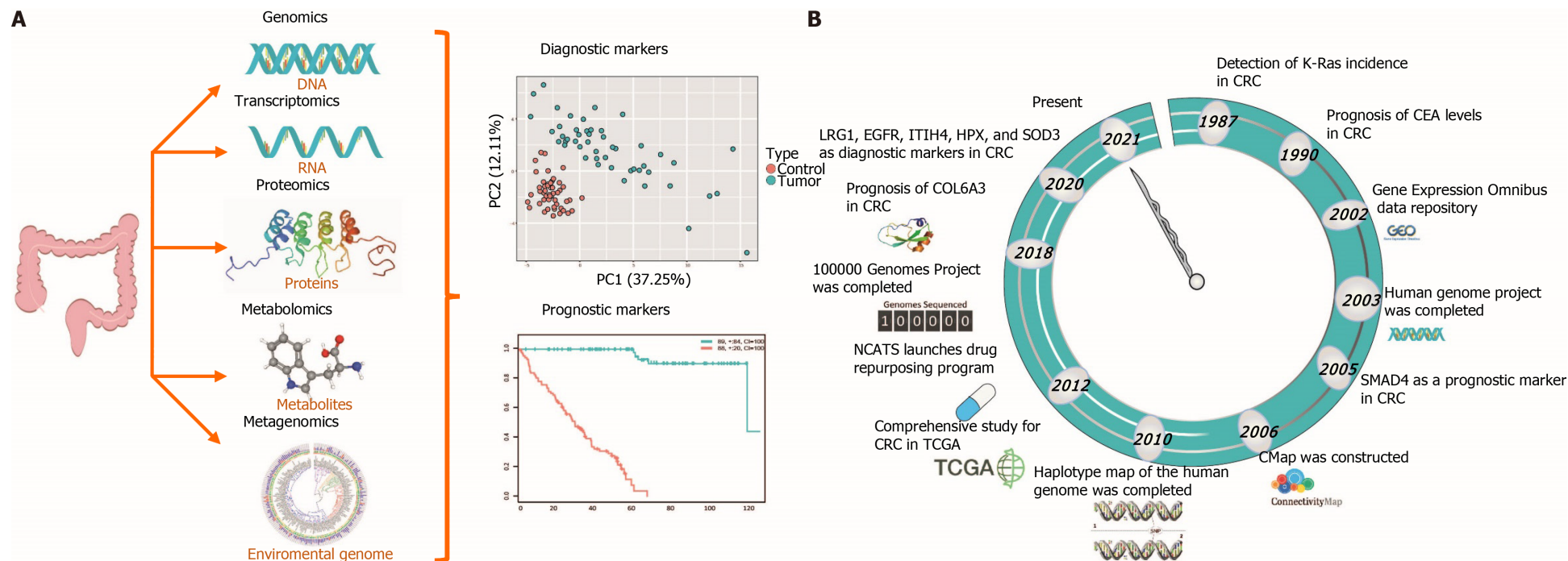


Figure 1 An overview of systems-level biomarkers. A: An overview of systems-level biomarkers in terms of diagnostics and prognostics from a variety of "omics" levels; B: Events recorded in history for omics and drug repositioning studies. LRG1: Leucine-rich alpha-2-glycoprotein 1; EGFR: Epidermal growth factor receptor; ITIH: Inter-alpha-trypsin inhibitor heavy chain family member 4; HPX: Hemopexin; SOD: Superoxide dismutase; CRC: Colorectal cancer; TCGA: The Cancer Genome Atlas; CEA: Carcinoembryonic antigen.

prerequisite.

Aberrant DNA methylation is a potential biomarker for the early detection of CRC. In one study, CpG methylation microarray analysis was performed in conjunction with a methylated DNA isolation assay to identify novel methylated genes at an early stage of CRC. Clinical validation tests showed that SDC2 had high sensitivity and specificity for detecting CRC, highlighting its diagnostic value as a blood-based biomarker for patients with CRC[20]. Li *et al*[21] identified a number of novel hypermethylated genes in CRC using the methylated-CpG island recovery assay in combination with microarrays. Of 211 hypermethylated candidate genes, only 3 novel hypermethylated genes, *PHOX1B*, *GAD2*, and *FGF12*, were selected for validation testing. These genes were better than *VIM* and *SEPT9* in discriminating CRC tissues compared to control tissues, indicating that they have the potential to be used as biomarkers for early diagnosis of CRC. Another comprehensive genomic study[22] performed a genome-wide search for hypermethylation events in primary CRC

compared to normal colonic tissue using microarrays. As a result of the systematic methylome-wide analysis, ten newly identified methylation events distinguished neoplastic and non-neoplastic colonic mucosa from CRC patients. Among the ten methylated events, VSX2 had the highest diagnostic accuracy.

Abnormal changes in the transcriptome occur as a result of epigenetic changes and loss of genomic stability in CRC[23,24]. An increasing number of studies have demonstrated that ncRNAs, the best-studied form of the RNA pool, are hallmarks of CRC, and their association with invasion and metastasis of CRC cells offers ncRNAs as promising new biomarkers for the early diagnosis and treatment of CRC[25]. Among ncRNAs, miRNAs are known to have a profound relationship with the stages and progression of CRC[26]. A comprehensive transcriptomic study was conducted by Yamada and colleagues using the RNA-seq approach to discover a novel lncRNA biomarker in CRC. They reported significant upregulation of four lncRNAs, CRCAL-4, CRCAL-3, CRCAL-2, and CRCAL-1, in patients with CRC[27]. Using the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes annotations, the expression of lncRNA NONHSAT074176.2 was found downregulated in CRC tissues, suggesting that it may be a potential diagnostic biomarker for CRC[28].

In addition to miRNA and lncRNA, circRNA is a new class of ncRNA that has emerged as a potential biomarker for various cancers including colorectal carcinogenesis. Li *et al*[29] identified several circRNAs that are significantly dysregulated in CRC tissue samples compared to adjacent normal mucosal tissues. Of the circRNAs analyzed, CircDDX17 showed strong potential as a diagnostic biomarker and therapeutic target for CRC.

Various proteomic approaches, mostly quantitative mass spectrometry (MS)-based technologies, have been used in the search for diagnostic biomarker candidates. To analyze the expression of proteins isolated from fresh-frozen human CRC tissue and the adjacent non-tumor tissue (12 patients), Ghazanfar *et al*[30] performed 2D PAGE coupled with MS. The results revealed a novel protein upregulated in CRC tissues, named ACTBL2. Hao and coworkers[31] examined 22 pairs of cancer tissues and adjacent healthy tissue samples collected from 22 participants using integrative proteomic analysis performed by high-resolution Fourier transform MS and revealed that DPEP1 was overexpressed in CRC tissues. Quesada-Calvo *et al*[32] examined 76 formalin-fixed paraffin-embedded (FFPE) colorectal tissue samples harvested from early CRC stages, including normal or inflamed mucosa, using label-free proteomics. Three biomarkers (KNG1, OLFM4, Sec24C) showed differences in expression levels in the early stages compared to normal and premalignant tissues. These results were verified by immunohistochemistry (IHC), although the experiment was conducted using liquid chromatography-tandem MS (LC-MS/MS). In another study, validation studies performed by Yamamoto *et al*[33] using FFPE CRC tissues showed that cyclophilin A, annexin A2, and aldolase A had high expression in cancerous tissues compared to non-cancerous tissues. A recent study identified seven potential biomarkers of CRC using differential expression analysis, systems biology, and proteomic analysis. These essential biomarkers, CALD1, CTNBNB1, CXCL14, PTCH1, CXCL8, TNFAIP3, and NNMT, are associated with other important target proteins such as APC, MAPK, and Gli[34].

Circulating biomarkers have great potential for early detection and clinical management of CRC, as they are cost-effective, easily accessible, minimally invasive, and low-risk[35]. In a prospective cross-sectional pilot study, Ivancic *et al*[36] demonstrated that a panel of five blood proteins (leucine-rich alpha-2-glycoprotein 1, Epidermal growth factor receptor, inter-alpha-trypsin inhibitor heavy chain family member 4, hemopexin, and superoxide dismutase 3) performed well for the detection of CRC using targeted LC-MS/MS. Validation tests showed that the panel has a specificity of 70% with a sensitivity of over 89% (Area under the curve = 0.86). Bhardwaj *et al*[37] performed LC/multiple reaction monitoring MS followed by proximity extension assay to identify a plasma protein panel. They showed a promising five-protein signature consisting of mannan-binding lectin serine protease 1, osteopontin (OPN/SPP1), serum paraoxonase lactonase 3, transferrin receptor protein 1, and amphiregulin for early detection of CRC.

In another study, Yu *et al*[38] analyzed 127 CRC serum samples and 90 healthy control samples and identified protein serine/threonine kinase 4 as a potential diagnostic biomarker for CRC using MS/MS. Fan *et al*[39] conducted a proteomic study to identify serum proteins by a combination of high-performance liquid chromatography and MS. They showed that macrophage mannose receptor 1 and S100 calcium-binding protein (S100A9) could be robust candidate biomarkers for adenomatous polyps and colorectal carcinomas. A quantitative proteomic study also detected a panel of protein biomarkers of adenomatous polyps and colorectal

carcinomas belonging to the serpin family, SERPINA1, SERPINA3, and SERPINC1, by multiplex quantification with an isobaric tag for relative and absolute quantification [40]. A study using label-free quantitative MS and protein microarray demonstrated adipophilin as a plasma biomarker to detect early-stage CRC [41].

Metabolomics studies focusing on disease-related metabolites allow clear differentiation of CRC patients from healthy controls, which is promising for the estimation of non-invasive biomarkers in the early diagnosis of CRC [42]. Fecal metabolic profiling studies provide important information to understand the details of CRC. To identify oncofetal diagnostic biomarkers, Ma *et al* [43] analyzed serum samples from CRC patients and healthy controls using an integrated proteomics and metabolomics approach. They detected ten candidate biomarkers consisting of 3-hydroxybutyric acid, L-valine, L-threonine, 1-deoxyglucose, glycine, MACF1, APOH, A2M, IGL, and VDB. In another metabolomics study on fecal samples, Lin *et al* [44] indicated that fecal metabolic profiles can differentiate between CRC patients and healthy controls, highlighting the potential utility of NMR-based fecal profiling for early detection in patients with CRC.

Serum NMR-based metabolic profiling ensures a substantial signature of CRC and has potential as a detection and diagnostic tool for patients with CRC. They showed that the rates of acetate/glycerol and lactate/citrate can be discriminatory biomarkers for colorectal polyps and CRC, respectively [45]. Another serum metabolome study was performed by Nishiumi *et al* [46] using gas chromatography (GC)/MS and they created a predictive model for early detection of CRC. Taurine, alanine, and 3-aminoisobutyrate showed discrimination between CRC patients in a recent study [47].

For metabolomic studies, urine samples may provide utility for the diagnosis of early and advanced stages of CRC. In an H-NMR-based metabolomic study, urine metabolites from CRC patients and healthy controls were profiled, including elevated acetoacetate, guanidinoacetate, cis-aconitate, trans-aconitate, glutamine, and homocysteine levels, as well as reduced creatinine, phosphorylcholine, dimethylsulfone, asparagine, alanine, isocitrate, hippurate, methylamine, cysteine, and phenylalanine levels [48]. A total of 16 promising urinary metabolites were detected to distinguish stage I/II CRC patients from healthy controls. In another NMR-based study, urine metabolomics profiles clearly and accurately distinguished CRC patients from healthy controls [47]. The results of the study showed that among the metabolites analyzed, only 6 metabolites significantly increased or decreased compared to healthy patients. Using capillary electrophoresis time-of-flight MS (CE-TOF/MS), Uchiyama *et al* [49] identified the interaction of CRC stages with the up- and down-regulation of various serum metabolites. They showed that benzoic acid might be a promising diagnostic biomarker for CRC patients. In a recent study, Liu *et al* [50] pointed out that miRNA and metabolite signatures have high diagnostic efficiency for CRC patients using two new methods, namely metabolomics based on GC/MS and serum miRNA detection. A study combining untargeted and targeted metabolomics showed that the differentiation of CRC patients from healthy controls revealed significant differences in serum concentrations of one endocannabinoid, two glycerophospholipids, and two sphingolipids [51].

In recent years, the number of metagenomic studies has gained significance, offering the opportunity to identify new diagnostic biomarkers for CRC clinical management. In Saudi Arabia, a comparative metagenomics study was conducted at a single center to understand the role of mucosal intestinal microbiota in CRC patients [52]. As a result of this study, among 11 genera found specific to CRC patients, *Bacteroides fragilis* and *Fusobacterium* were discovered to be present in the patient group compared to the control group. To identify the microbial composition of the microbiota in CRC, Kostic *et al* [53] performed whole-genome sequencing of nine CRC patients and healthy controls. This study revealed species-specific changes in the CRC microbiota, leading to diagnostic and clinical strategies for these patients. Using metagenomic data, Zeller *et al* [54] demonstrated that functional and taxonomic relationships with CRC and the ability to detect CRC determine early-stage disease from the fecal microbiota. While the metagenomics approach resolves associations between the gut microbiota and colorectal carcinogenesis, it holds promise for biomarker discovery for disease diagnosis and treatment. The biomarkers discovered in CRC are shown in Table 1.

The systems biology approach is a promising technology for the detection of novel diagnostic biomarkers in CRC. Despite the ever-increasing biomarker research based on systems biology, there is still a need to identify new biomarkers that have a crucial role in the early diagnosis and treatment of CRC, which are simple, inexpensive, and non-invasive, but have high sensitivity and specificity.

Table 1 Diagnostic biomarkers found in colorectal cancer

Diagnostic biomarker	Molecular level	Sample	Expression	Ref.
Actin Beta-Like 2	Protein	Fresh frozen tissue	↑	Ghazanfar <i>et al</i> [30], 2017
Dipeptidase 1	Protein	Fresh frozen tissue	↑	Hao <i>et al</i> [31], 2017
Olfamectomedin-4	Protein	FFPE tissue	↑	Quesada-Calvo <i>et al</i> [32], 2017
Kininogen-1				
Transport Protein Sec-24				
Cyclophilin A	Protein	FFPE tissue	↑	Yamamoto <i>et al</i> [33], 2016
Annexin A2				
Aldolase A				
Leucine-Rich Alpha-2 Glycoprotein 1	Protein	Serum	↑	Ivancic <i>et al</i> [36], 2020
Epidermal Growth Factor Receptor				
Hemopexin				
Superoxide Dismutase 3				
Inter-Alpha-Trypsin Inhibitor Heavy-Chain Family Member 4				
Mannan Binding Lectin Serine Protease 1	Protein	Plasma	-	Bhardwaj <i>et al</i> [37], 2019
Osteopontin				
Serum Paraoxonase Lactonase 3				
Transferrin Receptor Protein 1				
Amphiregulin				
Adipophilin	Protein	Plasma	-	Matsubara <i>et al</i> [41], 2011
Caldesmon 1	Protein	Fresh Frozen Tissue	-	Ilyas <i>et al</i> [34], 2020
Catenin Beta 1				
C-X-C Motif Chemokine Ligand 14				
Protein patched homolog 1				
Interleukin-8, Tumor necrosis factor-alpha induced protein 3, Nicotinamide N-methyltransferase				
Mitogen-activated protein kinase				
Adenomatous polyposis coli				
Zinc finger protein				
Serine/Threonine Kinase 4	Protein	Serum	↓	Yu <i>et al</i> [38], 2017
Macrophage mannose receptor 1	Protein		↑	Fan <i>et al</i> [39], 2016
S100 calcium binding protein				
Alpha-1-antitrypsin	Protein	Serum	↑	Peltier <i>et al</i> [40], 2016
Alpha-1-antichymotrypsin				
Anti- Thombin 3	Protein	Serum	↓	Peltier <i>et al</i> [40], 2016
3- Hydroxybutyric Acid		Serum	↑	Ma <i>et al</i> [43], 2012
Microtubule Actin Crosslinking Factor 1	Protein	Serum	↑	Ma <i>et al</i> [43], 2012
L-Valine	Amino acid	Serum	↓	Ma <i>et al</i> [43], 2012
L-Threonine				
1-Deoxyglucose				
Glycine				

Apolipoprotein H	Protein	Serum	↓	Ma <i>et al</i> [43], 2012
Alpha-2-Macroglobulin				
Immunoglobulin Lambda Locus				
Vitamin-D-binding protein				
Acetoacetate, Guanidinoacetate	Amino acid	Urine	↑/High	Wang <i>et al</i> [48], 2017
Cis-aconitate, Trans-aconitate, Glutamine				
Homocysteine				
Creatinine, Phosphorylcholine	Amino acid	Urine	↓/Low	Wang <i>et al</i> [48], 2017
Dimethyl sulfone, Asparagine, Alanine				
Isocitrate, Hippurate, Methylamine				
Cysteine, Phenylalanine				
Isoleucine, β-Hydroxybutyrate, lactate, acetate, glutamate, choline, glycine, serine, glucose	Amino acid	Biopsy	High	Nishiumi <i>et al</i> [46], 2012
Taurine, alanine, β -Aminoisobutyrate, valine	Amino acid	Urine	High	Kim <i>et al</i> [47], 2019
Threonine, glycerol, hippurate, ascorbate, creatinine and citrate	Amino acid	Urine	Less	Kim <i>et al</i> [47], 2019
Proline, succinate, isoleucine, leucine valine, alanine, glutamate, dimethylglycine and lactate	Amino acid	Fecal	High	Lin <i>et al</i> [44], 2019
Short Chain Fatty Acids, (acetate, propionate and butyrate), glucose, glutamine	Amino acid	Fecal	Less	Lin <i>et al</i> [44], 2019
Sphinganine, endocannabinoids		Serum	High	Martín-Blázquez <i>et al</i> [51], 2019
CRCAL-4, CRCAL-3, CRCAL-2 (Long Intergenic Non-Protein Coding RNA 858), and CRCAL-1	lncRNA	CRC cell line	↑	Yamada <i>et al</i> [27], 2018
NONHSAT074176.2	lncRNA	Fresh frozen tissue	↓	Zhang <i>et al</i> [28], 2018
CircDDX17	lncRNA	Fresh frozen tissue	Dysregulated	Li <i>et al</i> [29], 2018
Syndecan 2	Gene	Fresh frozen tissue	↑	Oh <i>et al</i> [20], 2013
PHOX1B, Glutamic acid de-carboxylase 2, and Fibroblast Growth Factor 12	Gene	Fresh frozen tissue	↑	Li <i>et al</i> [21], 2012
Visual System Homeobox 2	Gene	Neoplastic colonic tissue	↑	Mori <i>et al</i> [22], 2011

CRC: Colorectal cancer.

Prognostic markers

A prognostic biomarker is a biological characteristic that provides details on the patient's disease progression[55]. Prognostic biomarkers can be used to detect the progression of a pathological condition, including early recurrence and mortality[56, 57].

Recent studies have shown that the APC mutation is the most frequently seen mutation in CRC which has prognostic biomarker potential for clinical outcome in CRC[58]. Appropriate validation of this biomarker is needed to advance detection and better prognosis toward clinical outcome[59]. In a cohort genomic study, mutant TP53 status was associated with adjuvant 5-FU therapy in stage III CRC patients[60]. However, other research showed that mutant TP53 was significantly correlated with poor survival[61].

Micu *et al*[62] performed a retrospective study of 103 patients who had curative surgery for CRC, and they showed that the 5-year survival rate of patients with microsatellite instability (MSI) tumors was higher than that of the microsatellite stable tumor group. Another study showed that patients who had high MSI had longer disease-free survival (DFS) than patients with low MSI. This study shows that MSI has the potential to be a prognostic clinical parameter[63].

Mizuno *et al*[64] investigated the impact of SMAD4 gene mutation on clinical features and outcomes in patients undergoing liver resection for colorectal liver metastases using a next-generation somatic gene sequencing platform. In the

validation series, which included 237 patients, mutations in the *SMAD4* gene were correlated with a worse 3-year overall survival (OS) rate and were an autonomous predictor of worse OS. In a prospective study, multiple pan-cancer profiles of 33 Chinese mCRC patients were characterized utilizing extensive NGS. Further results showed that *SMAD4* and *NF1* mutations may be promising biomarker candidates for poor prognosis[65].

The impact of *BRAF* and *KRAS* on survival in stage II and III MSI colon cancer patients was investigated. *BRAF* and *KRAS* vs double wild-type mutations remained prognostic in stage II and III MSI colon cancer patients after multivariate analysis. These mutations should be analyzed if these genes are considered prognostic markers [66]. A survival study in patients with curatively resected stage I-III CRC demonstrated that the existence of *KRAF* and *BRAF* mutations was correlated with poor OS and DFS. In Japanese patients with successfully dissected CRC, *KRAS* and *BRAF* were associated with poorer survival, independent of MSI[67].

Nguyen *et al*[68] showed that the prognostic marker of 113 probe sets (CRC-113) was associated with disease incidence and survival in patients with CRC. Moreover, the CRC patients selected by the CRC-113 were able to benefit from postoperative chemotherapy, indicating that the CRC-113 gene signature could be a potential prognostic biomarker for CRC prediction.

High-throughput transcriptome studies provide significant opportunities to identify biomarkers that are effective for CRC prognosis. Zheng *et al*[69] indicated that MALAT1 lncRNA expression is upregulated in CRC tissues, as revealed by real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) analysis of 146 fresh tumor tissue samples. Therefore, a higher expression level of MALAT1 might be involved in CRC progression and therefore serve as a prognostic biomarker for patients with stage II/III CRC.

In another transcriptomic study, Ohtsuka *et al*[70] examined a large panel of lncRNA expression levels from CRC datasets of The Cancer Genome Atlas (TCGA) and recognized that H19 is the lncRNA most associated with OS of CRC patients. High expression levels of H19 have been related to tumor differentiation and advanced Tumor-Node-Metastasis stage[71] and may be a prognostic biomarker candidate for OS and DFS. A comprehensive study was conducted to reveal the role of 21 cancer-related lncRNAs in the prognosis of CRC using the PCR array. Their results showed that AFAP1-AS1, BCAR4, H19, HOXA-AS2, MALAT1, or PVT1 were upregulated, while ADAMTS9-AS2 was downregulated, and therefore seven lncRNAs have significant potential as a prognostic factor for CRC patients[72].

With regard to the discovery of novel miRNAs as biomarkers, recent studies suggested that miR-429 may be a novel independent biomarker for CRC prognosis[73, 74]. Sun and coworkers indicated that downregulation of miR-429 was significantly associated with poor prognosis for stage II/III colorectal carcinomas using RT-qPCR and tissue microarrays[74]. Another study focusing on miR-249 showed that overexpression of miR-249 correlated with a worse prognosis of CRC[73]. In a comprehensive study, Kandimalla *et al*[75] identified an 8-miRNA signature with high statistical significance consisting of hsa-mir-191, hsa-mir-200b, hsa-mir-30b, hsa-mir-30c2, hsa-mir-33a, has-mir-362, hsa-mir 429, and hsa-mir-744, representing a significantly improved prognostic potential for CRC patients. Løvf *et al*[76] recognized a novel CRC-specific transcript, VNN1-AB, from whole-transcriptome sequencing of seven CRC cell lines. This transcript had high sensitivity and complete specificity for CRC and therefore might be a potential prognostic factor for CRC.

The most widely used reliable prognostic protein biomarker in clinical practice is carcinoembryonic antigen (CEA), a high molecular weight glycoprotein secreted by 90% of CRCs. Although elevated CEA levels are associated with tumor progression, this is not specific to CRC, as they can also be caused by other conditions such as inflammatory bowel disease, liver disease, pancreatitis, and other malignancies[77-79]. In a recent study, Kirana *et al*[80] used a combination of laser microdissection (LM), 2D-DIGE, and MALDI-TOF MS methods to identify proteins that participate in the spread of CRC. First, cancer cells from patients with primary colorectal tumors at stage II were extracted into two groups using LM. Analysis of the isolated cancer cells showed an association of the expression of HLAB, 14-3-3b protein, ADAMTS2, LTB3, NME2, and JAG2 with tumor progression, invasion, and metastasis.

The collagen proteins are promising biomarker candidates, as the present study demonstrated that certain collagen proteins are upregulated in metastatic CRC. Comparative analysis of colorectal liver metastatic tissue with non-cancerous adjacent liver tissue using the MS-based proteomics approach has shown upregulation of 19 of 22 collagen chains. Further verification by IHC revealed that collagen type XII is significantly upregulated in CRC tissue[81].

In another study, Mori *et al*[82] used isobaric tags defined in the Isobaric Tags for Relative and Absolute Quantification (iTRAQ) methodology for proteomic analysis to determine novel biomarkers in lymph node metastases (LNM) in patients with CRC. The analysis revealed that 60 differentially expressed proteins were significantly related to LNM in CRC patients. Among these 60 proteins, the HSP47 protein was selected for detailed study as it has a general function and specific roles in the malignant phenotype. Validation analysis by IHC proved that HSP47 protein was highly expressed in CRC in contrast to adjacent healthy colonic mucosa. Another comparative proteomics study[83] was performed by the same group using the iTRAQ method. According to univariate and multivariate logistic analyses, the high expression level of ezrin protein was prognostic in CRC patients. Moreover, some studies have indicated that the higher expression level of Ezrin protein is associated with the aggressive behavior of tumors and poor prognosis of CRC[84,85].

Regarding the identification of robust biomarkers for CRC recurrence, a recent study[86] identified eight proteins as significant key markers with prognostic significance for tumor recurrence: Collagen VI, Forkhead Box O3, Inositol Polyphosphate-4-Phosphatase, LcK Tyrosine Kinase, Phospho-PEA15 (Ser116), Phospho-PRAS40, Rad51, and Phospho-S6 (Ser240-244). Snoeren *et al*[87] also demonstrated that higher expression of maspin is a prognostic biomarker for early recurrence in stage III and IV CRC patients.

Zhu *et al*[88] performed a case-control study based on magnet-based fractionation coupled with matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) to investigate serum samples from CRC patients. Using Fourier transform ion cyclotron resonance MS detection, peptides identified from a panel of proteins were alpha-fetoprotein, complement C4-A, fibrinogen alpha, the eukaryotic peptide chain-releasing factor GTP-binding subunit ERF3B, and angiotensinogen.

From a metabolomics perspective, there is an increasing number of studies focused on the discovery of biomarkers for CRC prognosis. In a prospective cohort study, Liesenfeld *et al*[89] analyzed the urinary metabolite profiling of CRC patients using GC-MS and ¹H NMR and showed that CRC patients before surgery can be distinguished from those after surgery. The metabolites analyzed were identified as prognostic biomarker candidates for the clinical management of CRC. Jiménez *et al*[90] also conducted a prospective study using high-resolution magic-angle spinning nuclear magnetic resonance spectroscopy (HR-MAS NMR) to analyze metabolomics profiles of CRC and adjacent macroscopically normal (or "off-tumor") mucosa harvested from the same resection specimen. In a large, four independent cohort study, Qiu *et al*[91] applied gas chromatography time-of-flight MS (GC-TOFMS) to analyze metabolites from CRC patients. They determined a distinctive metabolic signature with 15 significant biomarkers from CRC tissue samples, which has great prognostic and therapeutic potential. Chan *et al*[92] performed global metabolic profiling using HR-MAS NMR and GC/MS methodology to analyze metabolites of biopsied colorectal tumor samples and their matched normal mucosae. Their results demonstrated that unique metabolic signatures correlated with anatomic and clinicopathologic features of CRC, shedding light on providing novel phenotypic prognostic biomarkers for CRC management.

For the identification of reliable prognostic biomarkers, genomic studies provide an increasingly detailed and complex picture of the pathogenesis of CRC. The identification of novel prognostic biomarkers, their validation and translation into clinical application are very important in understanding the pathogenesis of CRC and to clarify issues such as distant metastasis and recurrence. Detailed information on prognostic biomarkers is provided in Table 2.

DR

DR from a systems biology perspective can be categorized as signature-based DR and network-based DR. Signature-based DR depends on gene expression signatures and compares between drug-gene and expression profiles of disease-gene[93,94]. Connectivity Map (CMap) is one of the most used tools which was established for signature-based approaches and it is a publicly available resource employed to detect small molecules and their mechanisms of action, chemicals or physiological processes, diseases, and drugs[95]. This approach is important for the discovery of new candidates and the experimental evaluation of computationally predicted candidates. Another category of DR is network-based DR, which is used to identify molecular mechanisms and key biomolecules in many diseases, including cancer, by creating

Table 2 Prognostic markers found in colorectal cancer

Prognostic biomarker	Molecular level	Sample	Expression	Ref.
Carcinoembryonic antigen	Protein	Plasma	↑	Li <i>et al</i> [79], 2018
Major histocompatibility complex, class I, B (HLAB)	Protein	Fresh Frozen Tissue	↑	Kirana <i>et al</i> [80], 2019
14-3-3b protein (YWHAB)				
A disintegrin and metalloproteinase with thrombospondin motifs 2				
Leukotriene B3				
Nucleoside Diphosphate Kinase 2				
Jagged Canonical Notch Ligand 2				
Collagen type XII (FACIT)	Protein	Colorectal liver metastasis tissue	↑	van Huizen <i>et al</i> [81], 2019
Heat shock protein 47	Protein	Fresh Frozen Tissue	↑	Mori <i>et al</i> [82], 2017
Ezrin protein	Protein	Cells and tissue	↑	Patara <i>et al</i> [85], 2011
Collagen VI (COL6)	Protein	Fresh Frozen Tissue	↑	Clarke <i>et al</i> [86], 2017
Forkhead box O3				
RAD51 (DNA repair protein RAD51 homolog 1)				
Lymphocyte-specific protein tyrosine kinase				
Inositol polyphosphate-4-phosphatase				
Phospho-PEA15 (Ser116)				
Phospho-S6 (Ser240-244)				
Phospho-PRAS40 (Thr-246)				
Mammary serine protease inhibitor	Protein	Fresh Frozen Tissue	↑	Snoeren <i>et al</i> [87], 2013
Alpha-fetoprotein	Protein	Serum	-	Zhu <i>et al</i> [88], 2013
Complement C4-A (C4A)				
Fibrinogen alpha				
Eukaryotic peptide chain release factor GTP-binding subunit ERF3B (GSPT2)				
Angiotensinogen				
Hippurate, Butyrate	Amino acid	Urine	↑	Liesenfeld <i>et al</i> [89], 2015
Glycerol, Galactarate				
Urea, Carnitine	Amino acid	Urine	↓	Liesenfeld <i>et al</i> [89], 2015
AFAP1 Antisense RNA 1	lncRNA	Datasets	↑	Li <i>et al</i> [72], 2016
Breast Cancer Anti-Estrogen Resistance 4				
H19				
HOXA Cluster Antisense RNA 2				
Metastasis Associated				
Lung Adenocarcinoma Transcript 1 (MALAT1)				
Plasmacytoma Variant Translocation 1				
ADAMTS9 Antisense RNA 2	lncRNA	Datasets	↓	Li <i>et al</i> [72], 2016
miR-429	microRNA	Fresh Frozen Tissue	↓	Li <i>et al</i> [73], 2013
hsa-mir-191, hsa-mir-200b	microRNA	Datasets	-	Kandimalla <i>et al</i> [75], 2018
hsa-mir-30b, hsa-mir-30c2				

hsa-mir-33a, hsa-mir-362				
hsa-mir-429, and hsa-mir-744				
VNN1-AB	Transcript	Datasets	-	Løvfi <i>et al</i> [76], 2014
Kirsten rat sarcoma oncogene 2 (KRAS)	Gene	Datasets	-	Kadowaki <i>et al</i> [67], 2015
Murine sarcoma viral oncogene homolog B1 (BRAF)				
Tumor protein 53	Gene	Datasets	-	Ting <i>et al</i> [59], 2013
SMAD Family Member 4	Gene	Datasets	-	Mei <i>et al</i> [65], 2018
Neurofibromatosis type 1				

disease-gene-drug triangles[94]. There are many examples of network-based pipelines and DR tools[96].

In this section, we have mentioned the drugs that have been repositioned using either network-based or signature-based DR strategies. However, we have categorized the drugs in relation to whether only computational prediction or *in vitro* assay has been carried out or clinical trials have been performed (Figure 2).

Computational predictions

Chung and collaborators presented a novel computational framework called Functional Module Connectivity Map for DR and used their framework for CRC. In their framework, the researchers first analyzed microarray data consisting of 32 CRC samples and 32 controls to find differentially expressed genes (DEGs). Then, gene-gene interaction networks were reconstructed around cancer and control samples, respectively. The gene-gene interaction networks were reduced to the function-function network using functional modules as nodes. Using the gene selection-by-trend-of-progression procedure, highly expressed hub genes in the function-function networks were identified and used as CMap query. CMap analysis culminated in several already known and effective CRC drugs. To validate the results of the CMap analysis, the researchers also performed cell viability assays to obtain eight candidates. As a result, the following drugs: GW-8510, ethacrynic acid, ginkgolide A, and 6-azathiymine were identified as drugs that inhibit CRC cells[97]. As the researchers demonstrated the upregulation of RRM2 in CRC by bioinformatics analysis, they sought to find a novel RRM2 inhibitor that has the potential to be used in CRC. To this end, the researchers first evaluated the expression of 3 RR subunits (RRM1, RRM2, and RRM2B) in cancer and normal cells using the Oncomine database and showed that the expression of RRM2 is upregulated in CRC. To further evaluate the effects of RRM2 on CRC, the researchers also analyzed microarray data (GSE8671 and GSE1710) and determined that RRM2 is a potential therapeutic target for CRC. After obtaining this information, the researchers attempted to find novel RRM2 inhibitors *via* CMap. They analyzed a microarray dataset (GSE15212) that included RRM2-knockdown SW480 human CRC cells and uncovered DEGs that were used as CMap queries. Four drugs (phenoxybenzamine, doxorubicin, daunorubicin, and GW-8510) with the highest CMap score were experimentally analyzed by cell viability assay and western blot analysis. The experiments showed that of the 4 drugs, GW-8510 prevented RRM2 expression. Thus, the study demonstrated the potential of GW-8510 as a CRC therapeutic agent targeting the inhibition of RRM2[98].

Another DR study used machine learning and molecular docking in colon cancer. For this purpose, the RNAseq data of colon adenocarcinomas were obtained from TCGA resource and the data were analyzed to find the DEGs. The significance of the DEGs was then validated *via* a machine learning approach and a total of 34 gene signatures were obtained. For the application of molecular docking, 34 gene signatures were converted into 3D structures and the list of FDA-approved anticancer drugs was used for docking (81 drugs). As a result of the molecular docking analyses, 4 targets emerged, namely GLTP, PTPRN, VEGFA, and FABP6. The 4 targets were investigated by literature search and both VEGFA and FABP6 were found to be upregulated in cancer cases; thus, they were considered potential targets. Docking studies showed that VEGFA and FABP6 had marked interactions with venetoclax and abemaciclib. However, the FABP6 gene has greater specificity for abemaciclib when compared with others. Therefore, this study suggested that abemaciclib targeting FABP6 has the potential to be a therapeutic target and needs further experimental validation[99]. Consequently, our research group applied a DR strategy by targeting the coexpression network of the protein Multidrug Resistance 1 (MDR1) expressed by the ABCB1

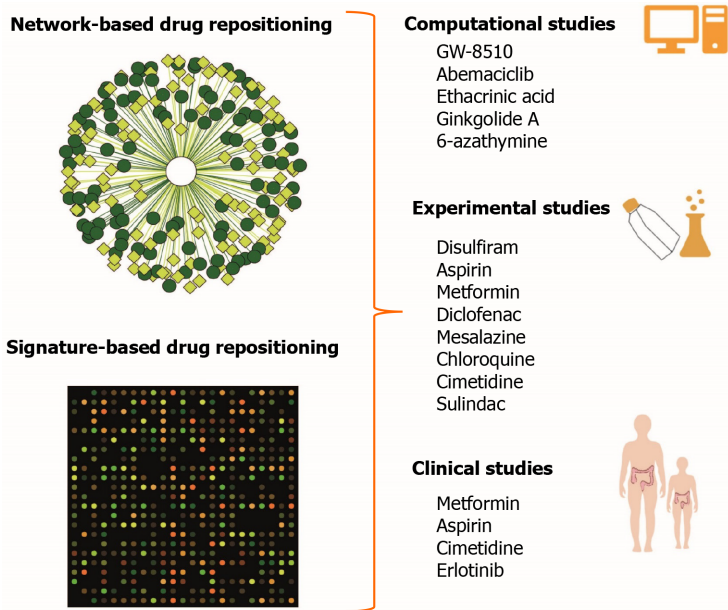


Figure 2 Drugs obtained via the drug repositioning approach in colorectal cancer.

protein that causes chemotherapy failure. To this end, we reconstructed four different coexpression networks around ABCB1 and exploited their prognostic and diagnostic capabilities in CRC. We performed DR using a reverse effect of coexpression signatures and estimated drug candidates by molecular docking in terms of determining the interaction potential between drug and MDR1. In addition, we carried out an *in silico* cross-validation study using transcriptome data for ABCB1-mediated co-expressed genes in drug-resistant HT29 cells. As a result of the study, we proposed drug candidates (*i.e.*, AG957, Ro-28-1675, Brazilin, Importazole, and PD407824) for CRC, especially by pointing out the importance of drug resistance in CRC[100] (Table 3).

Clinical and experimentally validated repurposed drugs

Metformin: Metformin is one of the most commonly used drugs in the treatment of type 2 diabetes mellitus. Metformin lowers blood glucose concentration without inducing hypoglycemia. As metformin is a readily available and inexpensive drug, numerous studies have been conducted on other potential indications[101]. In recent years, numerous *in vitro* and *in vivo* DR studies have shown that metformin can be used in the treatment or prevention of CRC. In an *in vivo* study, the effects of metformin treatment on diabetic rats were investigated. For this purpose, researchers injected STZ to induce diabetes in rats and injected 1,2-dimethylhydrazine to induce CRC. Then, 150 mg/kg metformin treatment was applied to rats once a week for 12 wk. Following metformin treatment and analysis of the results, it was shown that the number of precancerous lesions and cancer cell proliferation were reduced in rats [102]. In an *in vitro* study, HT29 cells were treated with metformin in a dose (0, 10 mmol/L/25 mmol/L/50 mmol/L) and time (24/48 h) dependent manner. The maximum dose used (50 mmol/L) for 48 h caused a maximum decrease (45%) in HT29 cells according to the proliferation index. Similarly, 60% of apoptotic cells were identified in the 50 mmol/L treatment for 48 h. Moreover, autophagic vacuoles were significantly conspicuous in metformin-treated cells at higher doses, whereas untreated cells showed a weak vacuole. In conclusion, the researchers indicated that metformin causes both apoptosis and autophagy of cultured HT29 cells[103]. A retrospective study based on a cohort of 1804 patients with diabetes and stage IV CRC showed that patients using metformin for their diabetes had better OS (hazard ratio = 0.85; 95% confidence interval = 0.76-0.94; *P* = 0.002) when considering their cancer status[104].

Aspirin: Due to its safety profile and widespread clinical use, aspirin is one of the best choices for drug repurposing. In a prospective cohort study, aspirin was shown to support the prevention of CRC. A total of 962 CRC cases were followed for 20 years, and patients were divided into aspirin users (325-mg tablets *per* week) and nonusers. The multivariate relative risk ratios with their 95% confidence intervals were 0.67-0.88

Table 3 Drug repurposing candidates for the prevention and/or treatment of colorectal cancer

Drug (s)	Pharmacological class	Original indication	CRC effect	Ref.
Based on computational approaches				
GW-8510, ethacrynic acid, ginkgolide A and 6-azathymine	GW-8510: Inhibitor of cyclin-dependent kinase-2; ethacrynic acid: Diuretic; ginkgolide A: Platelet-activating factor antagonist; 6-azathymine: D-3-aminoisobutyrate-pyruvate aminotransferase inhibitor	GW-8510: Prevention of chemotherapy-induced alopecia; ethacrynic acid: Treatment of high blood pressure and swelling; ginkgolide A: Treatment of a wide variety of cognitive and vascular disorders 6-azathymine: Has antibacterial and antiviral activities	Inhibit CRC cells	Chung <i>et al</i> [97], 2014
GW-8510	Inhibitor of cyclin-dependent kinase-2	Prevention of chemotherapy-induced alopecia	CRC therapeutic agent which targets RRM2 inhibition	Hsieh <i>et al</i> [98], 2016
Abemaciclib	Anti-neoplastic	Treatment of advanced or metastatic breast cancers	CRC therapeutic agent which targets FABP6	Liñares-Blanco <i>et al</i> [99], 2020
AG 957, Ro-28-1675, Brazilin, Importazole, PD 407824	AG 957: Inhibitor; Ro-28-1675: Activator; Brazilin: Anti-inflammatory agent; Importazole: Inhibitor; PD 407824: Indoles and derivatives	AG 957: Protein tyrosine kinase; Ro-28-1675: Glucokinase activator; Brazilin: NF-kappaB inhibitor and a hepatoprotective agent; Importazole: Transport receptor importin-β; PD 407824: Wee1/Chk1 inhibitor	CRC drug candidates	Beklen <i>et al</i> [100], 2020
Based on clinically or experimentally validated approaches				
Metformin	Antidiabetic agent	Treatment of type 2 diabetes mellitus	Reduces pre-cancerous lesions and cancerous cell proliferation Results in better overall survival Causes both apoptosis and autophagy of cultured HT29 cells	Jia <i>et al</i> [102], 2015 Bishnoi <i>et al</i> [104], 2018 Sena <i>et al</i> [103], 2018
Aspirin	Nonsteroidal anti-inflammatory	Relieves minor aches, pains, and fevers	Regular, long-term aspirin use reduces CRC risk Reduces CRC cell proliferation Daily use for about 5 yr is efficient in prevention, with a latency time of about 10 yr Restrains CRC tumor metastasis	Chan <i>et al</i> [105], 2005 Reddy <i>et al</i> [106], 2006 Flossmann <i>et al</i> [107], 2007 Jin <i>et al</i> [108], 2019
Cimetidine	Gastrointestinal agent	Treatment of ulcers and gastroesophageal reflux disease	Short course of cimetidine treatment has an impact on patient survival Inhibits the adhesion of CRC cells and represses spread of cancer cells Reduces frequency of metastasis and increases survival rate Prolongs the probability of recurrence	Kelly <i>et al</i> [110], 1999 Kobayashi <i>et al</i> [111], 2000 Matsumoto <i>et al</i> [112], 2002 Ali <i>et al</i> [113], 2018
Diclofenac	Nonsteroidal anti-inflammatory	Relieves pain and inflammation	Contributes to tumor growth inhibition in colon cancer Promotes cell death	Falkowski <i>et al</i> [116], 2003 Arisan <i>et al</i> [117], 2018
Chloroquine	Antimalarial	Treatment of malaria	Increases cell inhibition and decreases reproduction	Sasaki <i>et al</i> [119], 2010

			Use at high doses (40–160 µM) encourages lysosomal membrane permeabilization and cell death	Park <i>et al</i> [120], 2014
Sulindac	Nonsteroidal anti-inflammatory	Reduces pain, swelling, and joint stiffness due to arthritis	Decreases final tumor counts and prevents CRC especially when given in intermittent doses	Chandra <i>et al</i> [122], 2017
			Decreases polyp count	Davis <i>et al</i> [123], 2020
Mesalazine	Anti-inflammatory	Treatment of mild to moderate ulcerative colitis	Decreases CRC cell number and enhances apoptosis	Reinacher-Schick <i>et al</i> [125], 2003
			Causes CRC cell cycle arrest and cell death	Koelink <i>et al</i> [126], 2010
Disulfiram	Enzyme inhibitor	Alcohol addictive disorder	Promotes cell viability inhibition	Stenvang <i>et al</i> [128], 2018
Erlotinib	Anti-neoplastic	Treatment of non-small cell lung and pancreatic cancer	Contributes to enhanced progression-free survival and overall survival of metastatic CRC patients	Shi <i>et al</i> [130], 2017

CRC: Colorectal cancer.

for aspirin users compared with nonusers. Thus, regular, long-term aspirin use reduced the risk of CRC[105]. In another *in vivo* study which evaluated the efficacy of aspirin on CRC, researchers first induced CRC in rats using azoxymethane. They then fed the rats high-dose aspirin (400 ppm) for 42 wk. According to the culminated results, the researchers reported that 400 ppm aspirin significantly prevented the occurrence of CRC by about 29% ($P = 0.05$). Therefore, the study concluded that aspirin has the ability to reduce cell proliferation in CRCs[106]. Two large randomized trials comparing aspirin users (300 mg, 500 mg, or 1200 mg) and non-users were followed up for more than 20 years to find an association between aspirin and CRC risk. After evaluating the results, it was found that daily aspirin use (300 mg or more) for a period of about 5 years was efficient in preventing CRC, with a latency period of about 10 years[107]. SW480 colon cancer cells were treated with 0.5-10 mmol/L aspirin for 2 d to study the effects of aspirin on colon cancer. Aspirin was shown to inhibit colon cancer cell migration by regulating epithelial-mesenchymal transition and it was emphasized that aspirin has the ability to curb tumor metastasis[108].

Cimetidine: The histamine receptor 2 antagonist cimetidine has intense immunomodulatory effects on the innate and adaptive immune systems[109]. The anticancer effects of cimetidine have been highlighted in many cancers, including CRC. To investigate the effects of cimetidine on colon carcinomas, 125 patients who planned to undergo elective colon or rectal excision were treated with cimetidine for 5 d before surgery. Using IHC, Kaplan-Meier, and computer video image analysis, the patients who received 800 mg of cimetidine twice daily were compared with the placebo group. Based on the results obtained, the researchers concluded that a short course of cimetidine treatment before surgery may have an impact on patient survival[110]. Another study by Kobayashi *et al*[111] investigated the mechanism of cimetidine in CRC. According to the analyses, they showed that cimetidine could inhibit adhesion of the CRC cell line to endothelial cells. Similarly, cimetidine can suppress the spread of cancer cells in a mouse model by decreasing the cell surface expression of E-selectin (an adhesion molecule) on endothelial cells. The effect of cimetidine on survival was studied using a total of 64 CRC-operated patients. Two weeks after surgery, patients were treated with 800 mg of cimetidine along with 200 mg of 5-FU (34 patients), while the placebo group (30 patients) received only 5-FU for one year. After treatment, the 10-year cumulative survival rate was 84.6% in the cimetidine group and 49.8% in the control group. The researchers also indicated that cimetidine reduced the incidence of metastasis in CRC patients. Finally, by immunostaining tumor tissue, the researchers showed that CRC patients who had high sLx or sLa epitope expression on tumor cells had an increased survival rate[112]. Another recent study investigated whether the time to CRC relapse can be prolonged with cimetidine. The clinical outcomes of a total

of 38 patients (stage III) were followed according to whether they received cimetidine for more than 2 years. Clinical outcomes were compared between the groups using univariate analysis and Kaplan-Meier modeling. It was found that days to relapse were significantly longer in the chemotherapy/cimetidine group than in the chemotherapy alone group. In addition, they reported that there was a direct association between the duration of cimetidine use and total cumulative cimetidine dose and cancer survival[113].

Diclofenac: The nonsteroidal anti-inflammatory drug diclofenac inhibits the enzyme cyclooxygenase-2 and has analgesic and antipyretic effects in addition to its anti-inflammatory function[114]. With increasing interest in the use of diclofenac in oncology, evidence of the anticancer effect of diclofenac in various cancers, including CRC[115], is increasing day by day. One study investigated the effect of diclofenac on the growth of murine C-26 colon carcinoma cells and C-26 tumors in syngeneic mice. For this purpose, the researchers fed the mice (which were implanted with colon tumors) diclofenac at a dose of 250 mg/L for 12 d. As a result of diclofenac treatment, cell death occurred on C-26 cells in a dose-dependent manner. In addition, diclofenac contributed to the inhibition of tumor growth in mice. Overall, the study suggested that diclofenac was a potential therapeutic and protective agent for colon cancer[116]. To reveal the molecular and therapeutic targets of diclofenac in colon cancer cells, the researchers used diclofenac in two cell types, HCT 116 (wt p53) and SW480 (mutant p53R273H). The results of the analysis culminated in subcytotoxic concentrations of diclofenac (400 μ M) causing an increase in cell death in HCT 116 cells compared to SW480 cells. Furthermore, diclofenac promotes cell death by altering the PI3K/Akt/MAPK signaling axis in HCT 116 colon cancer cells[117].

Chloroquine: The main use of chloroquine is in the treatment of malaria. However, DR studies showed that the use of chloroquine is also effective in cancer treatment strategies, especially in combination with preferred anti-cancer agents[118]. In another study, the human CRC cell line HT29 was treated with chloroquine and/or 5-FU to investigate whether chloroquine potentiates the effect of 5-FU in CRC. For this purpose, cells were treated with chloroquine at different doses (0.1, 1, 10, 100, or 1000 μ M) for 12 and 24 h, and treated with 5-FU at doses of 0.01, 0.1, 1, 10, 100, or 1000 μ M for 24, 48, and 72 h. Cells were also first treated with chloroquine (80 μ M) for 12 h and then 5-FU. The researchers found that chloroquine co-treatment caused an increase in cell inhibition and a decrease in reproduction of about 33% compared to the control group. Accordingly, the researchers concluded that combination therapy with chloroquine and 5-FU is an impressive and promising strategy for curing CRC[119]. The effects of different concentrations of chloroquine on CRC were investigated using the HCT15 cell line. For this purpose, the researchers treated the cell lines with chloroquine at different doses (5 μ M to 80 μ M) in a time-dependent manner (12, 24, 48 or 72 h). They also used the anticancer drug RNVP-BEZ235, which is an inhibitor of PI3K and mTOR, to study the different effects of chloroquine. It was found that chloroquine had different effects depending on the dose used. For example, chloroquine at low doses (10-20 μ M) rescues cell viability and acts as an autophagy inhibitor when used alone or with NVP-BEZ235. In contrast, chloroquine at high doses (40-160 μ mol/L) promotes lysosomal membrane permeabilization and cell death[120].

Sulindac: The non-steroidal anti-inflammatory drug, sulindac, is defined as a cyclooxygenase inhibitor. As cell death and proliferation of cancer cell lines can be affected by a cyclooxygenase-independent mechanism, sulindac has been described as a promising anti-cancer repurposing agent[121]. In a study aimed at evaluating the dose effects of sulindac on CRC prevention, four different dosing regimens were applied to azoxymethane-treated mice. In the first dosing regimen, the researchers treated the mice with sulindac daily for 20 wk. In the second dosing regimen, the mice were treated with sulindac for 2 wk, treatment was discontinued for the next 2 wk, and this process was continued 5 times. In the third dosing regimen, sulindac was given to mice for 10 wk and then not given for 10 wk. A treatment regimen was not used in the last group and this was considered the control group. The first two sulindac regimens caused markedly lower final tumor counts compared to controls ($P = 0.001$). In addition, there was a significant decrease in tumor burden in all treatment groups compared with the control group. The study concluded that sulindac is a potential agent in the prevention of CRC when used especially in intermittent doses [122]. To investigate the effects of sulindac on CRC, a recent study treated C57B/6 Apcmin/+ mice with sulindac (30 mg/kg) for 3 wk. Moreover, the study hypothesized that the presence of phosphatidylcholine (PC) in sulindac treatment does not cause

gastrointestinal ulceration and bleeding, making use of the drug more convenient for the general population. Therefore, in addition to sulindac treatment, the researchers also treated mice with sulindac-PC (30 mg/kg). The results showed that treatment with sulindac or sulindac-PC significantly affected the polyp count. Sulindac treatment resulted in a 58% reduction in polyp count, while sulindac-PC resulted in a 64% reduction. In addition, the researchers confirmed their hypothesis and showed that treatment with sulindac-PC minimized gastrointestinal ulceration compared to treatment with sulindac alone[123].

Mesalazine: Mesalazine, also known as 5-aminosalicylic acid, is an anti-inflammatory drug used to treat patients with mild to moderate ulcerative colitis[124]. Since the risk of developing CRC is increased in patients with ulcerative colitis, researchers investigated whether this drug could be used for both therapeutic purposes. To evaluate the effects of mesalazine in CRC, HT29 CRC cells were treated with mesalazine at various concentrations for 3 d. It was found that mesalazine decreased cell proliferation and increased apoptosis of CRC cells. Treatment with mesalazine at a concentration of 20 mmol/L significantly decreased the number of CRC cells compared to controls. In addition, 30 mmol/L mesalazine decreased the number of HT29 cells by 50%. Increasing the concentration of mesalazine from 0 to 40 mmol/L also increased apoptosis. From these findings, the researchers concluded that mesalazine is a candidate drug for the prevention of CRC[125]. In another study, the HT29, Caco2, and HCT-116 cell lines were treated in a time- (24, 48, and 72 h) and dose- (0-50 mmol/L) dependent manner. It was revealed that 20 mmol/L or higher mesalazine concentrations significantly decreased cell proliferation at 72 h. Also, mesalazine concentrations above 35 mmol/L decreased cell proliferation even to baseline levels at 24 h. The researchers suggested that mesalazine causes cell cycle arrest and cell death in both a dose- and time-dependent manner, from which the researchers concluded that mesalazine may contribute to CRC prevention or treatment[126].

Disulfiram: Disulfiram is used to treat alcohol dependence[127]. To understand the effects of disulfiram in CRC, researchers applied disulfiram to parental colorectal cell lines as well as cell lines resistant to either oxaliplatin or SN-38. The addition of metal ions, particularly copper, to disulfiram is known to support and enhance the anti-cancer effects of disulfiram. Therefore, the researchers also combined disulfiram with copper and applied it to all cell lines. According to the cell viability assay, the combination of disulfiram with copper promoted the inhibition of cell viability. Moreover, the combination of disulfiram and copper with oxaliplatin or SN-38 resulted in inhibitory effects on the relevant resistant cell lines studied[128].

Erlotinib: Erlotinib is an antineoplastic agent used to treat non-small cell lung and pancreatic cancer[129]. A study conducted by Shi *et al*[130] investigated the effect of erlotinib on CRC. The study included one hundred and thirty-two patients diagnosed with metastatic CRC who received the drug. Patients were divided into two groups depending on the drug regimen used. In the first group, patients were treated with bevacizumab and FOLFOX4 in 2-wk cycles, while in the second group they took erlotinib (100 mg) daily in addition to the bevacizumab-FOLFOX4 treatment combination. Patients were followed up for 3 years. Overall, the combination of the three drugs (bevacizumab, FOLFOX4, and erlotinib) improved progression-free survival and OS of metastatic CRC patients. A summary of the mentioned repurposed drugs is provided in Table 3.

CONCLUSION

In order to provide an updated vision of the key insights into CRC, we specifically reviewed systems biology studies on CRC. The incidence and mortality rates of CRC are high and more effective treatment options are required. Although most of the major cancer genes involved in CRC have been well characterized, the influence of additional factors in this disease remains undefined. Thus, the integration of different omics studies may provide new opportunities to reveal the mechanism of CRC. Parallel to the improvement in the molecular biology and systems biology techniques, more specific and sensitive biomarkers will improve the diagnosis of CRC at the early stages and hence, the prognosis of thousands of patients. Recent studies on CRC have focused on microbiota not only as biomarkers but also as a therapy option by improving drug response to traditional chemotherapeutics. Controlling microbiota

may also reduce dosages and the rate of drug administration resulting in improvement in the patient's life quality. Although different omics studies from non-coding genes to metabolites have shown promising results which may be translated into the clinic in the foreseeable future, there is an urgent need for validation in larger populations. As with other cancers, an extensive part of research is based on drug discovery and the development of new therapies. In this study, we feature DR rather than drug discovery due to its many advantages. The less aggressive and more effective drugs may be redirected for CRC treatment *via* rational DR studies. In the future, repositioned drugs and their clinical implementation will increase the OS and quality of life of CRC patients.

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